66/60/90

Jc672 U.S. PTO

A + BOX SEQ

PATENT/Docket No.: **6142.N2 CP**

CERTIFICATE OF MAILING (37 CFR 1.10)

"Express Mail" No: EM177638640 US **Date of Deposit:** June 9, 1999

I hereby certify that this transmittal together with the patent application referred to below is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, DC 20231.

Julie K. Lyons, Legal Assistant — Julie K Lyons

Name of Person Mailing Paper **Signature**

Jc530 U.S. PTO 09/328877 06/09/99

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner of Patents and Trademarks
Washington, DC 20231

TRANSMITTAL OF A NON-PROVISIONAL APPLICATION Under 37 CFR 1.53(b)(1)

Sir:

Transmitted herewith for filing is the patent application entitled Human Sel-10 Polypeptides and Polynucleotides that Encode Them.

[X] This application is being mailed by Express Mail under 37 CFR 1.10 and the required certificate appears above.

[] The Oath or Declaration required under 37 CFR 1.63 **is being transmitted** with this application.

[x] The Oath or Declaration required under 37 CFR 1.63 **is not being transmitted** with this application pursuant to 37 CFR 1.53(f).

[x] A disk containing nucleotide and/or amino acid sequences in a computer readable format is attached. The contents of the sequence listing in the application is the same as the document on the disk. (37 CFR 1.821(f) and MPEP 2422.06)

The filing fee has been calculated as shown below:

	Total No. of Claims	No. of Claims Without Additional Fee	Excess Claims	$ Rate	Fee
Total Claims Fee	**41**	20	21	x 18	378
Independent Claims Fee	**2**	3		x 78	0
Multiple Dependent Claim	0			x 260	0
Basic Fee					760
Total Filing Fee					$ 1138

[X] **SPECIFIC DEPOSIT ACCOUNT AUTHORIZATION.** Please charge my Deposit Account No. **21-0718** in the amount of the total filing fee above. Triplicate copies of this sheet are enclosed.

[X] **GENERAL DEPOSIT ACCOUNT AUTHORIZATION.** The Commissioner is hereby authorized to charge payment of the following fees associated with this communication or during the pendency of this application or credit any overpayment to Deposit Account No. **21-0718**.

(1) Any additional filing fees or fees for the presentation of additional claims required under 37 CFR 1.16.

(2) Any patent application processing fees under 37 CFR 1.17.

No authorization is given to charge the Issue Fee (37 CFR 1.18).

[ ] **ASSIGNMENT RECORDAL DEPOSIT ACCOUNT AUTHORIZATION.** Please charge my Deposit Account No. **21-0718** in the amount of **$40.00** for recordal for the attached assignment of the invention to Pharmacia & Upjohn Company. A separate cover sheet for assignment accompanying a new patent application is also attached.

This application is being made, or is authorized to be made, by the inventor(s) as set forth on the attached Inventor Information Sheet. The person or persons listed are believed to be the original, first and sole inventor (if only one name is listed on the attached Inventor Information Sheet) or original, first and joint inventor (if plural names are listed on the attached Inventor Information Sheet) of the subject matter which is claimed and for which a patent is sought.

The undersigned hereby requests that all correspondence and telephone communications in connection with this application be directed to the undersigned person at the mailing address and telephone number shown below.

Respectfully submitted,

Lori Kerber Date: June 9, 1999

Lori L. Kerber, Attorney
Registration No. 41,113

Pharmacia & Upjohn Company
Global Intellectual Property
301 Henrietta Street
Kalamazoo, Michigan 49001

Telephone No. (616) 833-0974 or (616) 833-9500
Telefax No. (616) 833-8897 or (616) 833-2316

Enclosures:

[X] Patent Application
[] Declaration (37 CFR 1.63) and Power of Attorney
[x] Disk containing Nucleotide and/or amino Acid Sequence Listing
[X] Return Post Card

INVENTOR INFORMATION SHEET

Full name of sole or first inventor:	Mark E. Gurney
Address:	910 Rosewood Avenue, SE Grand Rapids, Michigan 49506
Citizenship:	USA
Full name of second inventor:	Adele M. Pauley
Address:	213 East Brighton Plainwell, Michigan 49080
Citizenship:	USA
Full name of third inventor:	Jinhe Li
Address:	8548 Old Oak Circle Kalamazoo, Michigan 49009
Citizenship:	People's Republic of China

Human Sel-10 Polypeptides and Polynucleotides that Encode Them

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of the following provisional application: U.S. Serial No. 60/068,243, filed 19 December 1997, under 35 USC 119(e)(1). This application also claims the benefit of the following application: U.S. Serial No.09/213,888, filed 17 December 1998, under 35 USC 120.

FIELD OF THE INVENTION

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding either of two alternative splice variants of human sel-10, one of which is expressed in hippocampal cells, and one of which is expressed in mammary cells. The invention also provides isolated sel-10 polypeptides.

BACKGROUND OF THE INVENTION

Alzheimer's disease (AD) is a degenerative disorder of the central nervous system which causes progressive memory and cognitive decline during mid to late adult life. The disease is accompanied by a wide range of neuropathologic features including extracellular amyloid plaques and intra-neuronal neurofibrillary tangles. (Sherrington, R., *et al.; Nature 375*: 754-60 (1995)). Although the pathogenic pathway leading to AD is not well understood, several genetic loci are known to be involved in the development of the disease.

Genes associated with early onset Alzheimer's disease (AD) have been identified by the use of mapping studies in families with early-onset AD. These studies have shown that genetic loci on chromosomes 1 and 14 were likely to be involved in AD. Positional cloning of the chromosome 14 locus identified a novel mutant gene encoding an eight-transmembrane domain protein which subsequently was named presenilin-1 (PS-1). (Sherrington, R., *et al.; Nature 375*: 754-60 (1995)). Blast search of the human EST database revealed a single EST exhibiting homology to PS-1, designated presenilin-2 (PS-2) which was shown to be the gene associated with AD on chromosome 1. (Levy-Lahad, E. *et al., Science 269*:973-977 (1995); Rogaev, E. I., *et al., Nature 376*: 775-8 (1995); Li, J. *et al., Proc. Natl. Acad. Sci. U.S.A. 92*: 12180-12184 (1995)).

Mutations in PS-1 and PS-2 that are associated with Alzheimer's disease are primarily missense mutations. Both PS-1 and PS-2 undergo proteolytic processing, which can be altered by the point mutations found in familial Alzheimer's disease [Perez-Tur, J. *et al., Neuroreport 7*: 297-301 (1995); Mercken, M. *et al., FEBS Lett. 389*: 297-303 (1996)]. PS-1 gene expression is widely distributed across tissues, while the highest levels of PS-2 mRNA are found in pancreas and skeletal muscle. (Li, J. *et al., Proc. Natl. Acad. Sci. U.S.A. 92*: 12180-12184 (1995); Jinhe Li, personal communication). The highest levels of PS-2 protein, however, are found in brain (Jinhe Li, personal communication). Both PS-1 and PS-2 proteins have been localized to the endoplasmic reticulum, the Golgi apparatus, and the nuclear envelope. (Jinhe Li, personal communication; Kovacs, D.M. *et al., Nat. Med. 2*:224-229 (1996); Doan, A. *et al., Neuron 17:* 1023-1030 (1996)). Mutations in either the PS-1 gene or the PS-2 gene alter the processing of the amyloid protein precursor (APP) such that the ratio of A-beta$_{1-42}$ is increased relative to A-beta$_{1-40}$ (Scheuner, D. *et al., Nat. Med. 2*: 864-870 (1996)). When coexpressed in transgenic mice with human APP, a similar increase in the ratio of A-beta$_{1-42}$ as compared to A-beta$_{1-40}$ is observed (Borchelt, D. R. *et al., Neuron 17*: 1005-1013 (1996); Citron, M. *et al., Nat. Med. 3*: 67-72 (1997); Duff, K. *et al., Nature* 383: 710-713 (1996)), together with an acceleration of the deposition of A-beta in amyloid plaques (Borchelt *et al., Neuron 19:* 939 (1997).

Despite the above-described observations made with respect to the role of PS-1 and PS-2 in AD, their biological function remains unknown, placing them alongside a large number of human disease genes having an unknown biological function. Where the function of a gene or its product is unknown, genetic analysis in model organisms can be useful in placing such genes in known biochemical or genetic pathways. This is done by screening for extragenic mutations that either suppress or enhance the effect of mutations in the gene under analysis. For example, extragenic suppressors of loss-of-function mutations in a disease gene may turn on the affected genetic or biochemical pathway downstream of the mutant gene, while suppressers of gain-of-function mutations will probably turn the pathway off.

One model organism that can be used in the elucidation of the function of the presenilin genes is *C. elegans*, which contains three genes having homology to PS-1 and PS-2, with *sel*-12 having the highest degree of homology to the genes encoding the human presenilins. *Sel*-12 was discovered in a screen for genetic suppressers of an activated notch receptor, *lin*-12(d) (Levitan, D. *et al., Nature 377:* 351-354 (1995)). *Lin*-12 functions in development to pattern cell lineages. Hypermorphic mutations such as *lin*-12(d), which increase *lin*-12 activity, cause a "multi-vulval" phenotype, while hypomorphic mutations which decrease activity cause eversion of the vulva, as well as homeotic changes in several other cell lineages (Greenwald, I., *et al., Nature 346*: 197-199 (1990); Sundaram, M. *et al., Genetics 135*: 755-763 (1993)). *Sel*-12 mutations suppress hypermorphic *lin*-12(d) mutations, but only if the *lin*-12(d) mutations activate signaling by the *intact* lin-12(d) receptor (Levitan, D. *et al., Nature 377:* 351-354 (1995)). *Lin*-12 mutations that truncate the cytoplasmic domain of the receptor also activate signaling (Greenwald, I., *et al., Nature 346*: 197-199 (1990)), but are not suppressed by mutations of *sel*-12 (Levitan, D. *et al., Nature 377:* 351-354 (1995)). This implies that *sel*-12 mutations act upstream of the *lin*-12 signaling pathway, perhaps by decreasing the amount of functional *lin*-12 receptor present in the plasma membrane. In addition to suppressing certain *lin*-12 hypermorphic mutations, mutations to *sel*-12 cause a loss-of-function for egg laying, and thus internal accumulation of eggs, although the mutants otherwise appear anatomically normal (Levitan, D. *et al., Nature 377:* 351-354 (1995)). *Sel*-12 mutants can be rescued by either human PS-1 or PS-2, indicating that *sel*-12, PS-1 and PS-2 are functional homologues (Levitan, D., *et al., Proc. Natl. Acad. Sci. U.S.A 93*: 14940-14944 (1996)).

A second gene, *sel*-10, has been identified in a separate genetic screen for suppressors of *lin*-12 hypomorphic mutations. Loss-of-function mutations in *sel*-10 restore signaling by *lin*-12 hypomorphic mutants. As the lowering of sel-10 activity elevates lin-12 activity, it can be concluded that sel-10 acts as a negative regulator of lin-12 signaling. Sel-10 also acts as a negative regulator of sel-12, the *C. elegans* presenilin homologue (Levy-Lahad, E. *et al., Science 269*:973-977 (1995)). Loss of sel-10 activity suppresses the egg laying defect associated with hypomorphic mutations in *sel*-12 (Iva Greenwald, personal communication). The effect of loss-of-function mutations to *sel*-10 on *lin*-12 and *sel*-12 mutations indicates that sel-10 acts as a negative regulator of both lin-12/notch and presenilin activity. Thus, a human homologue of *C. elegans* sel-10 would be expected to interact genetically and/or physiologically with human presenilin genes in ways relevant to the pathogenesis of Alzheimer's Disease.

In view of the foregoing, it will be clear that there is a continuing need for the identification of genes related to AD, and for the development of assays for the identification of agents capable of interfering with the biological pathways that lead to AD.

INFORMATION DISCLOSURE

Hubbard EJA, Wu G, Kitajewski J, and Greenwald I (1997) Sel-10, a negative regulator of lin-12 activity in Caenorhabditis elegans, encodes a member of the CDC4 family of proteins. Genes & Dev 11:3182-3193.

Greenwald-I; Seydoux-G (1990) Analysis of gain-of-function mutations of the *lin-12* gene of *Caenorhabditis elegans. Nature. 346*: 197-9

Kim T-W, Pettingell WH, Hallmark OG, Moir RD, Wasco W, Tanzi R (1997) Endoproteolytic cleavage and proteasomal degradation of presenilin 2 in transfected cells. J Biol Chem 272:11006-11010.

Levitan-D; Greenwald-I (1995) Facilitation of *lin-12*-mediated signalling by *sel-12*, a *Caenorhabditis elegans* S182 Alzheimer's disease gene. *Nature. 377*: 351-4.

Levitan-D; Doyle-TG; Brousseau-D; Lee-MK; Thinakaran-G; Slunt-HH; Sisodia-SS; Greenwald-I (1996) Assessment of normal and mutant human presenilin function in *Caenorhabditis elegans. Proc. Natl. Acad. Sci. U.S.A. 93*: 14940-4.

Sundaram-M; Greenwald-I (1993) Suppressors of a *lin-12* hypomorph define genes that interact with both *lin-12* and glp-1 in *Caenorhabditis elegans. Genetics. 135*: 765-83.

Sundaram-M; Greenwald-I (1993) Genetic and phenotypic studies of hypomorphic *lin-12* mutants in *Caenorhabditis elegans. Genetics. 135*: 755-63.

F55B12.3 GenPep Report (WMBL locus CEF55B12, accession z79757).

WO 97/11956

SUMMARY OF THE INVENTION

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding human sel-10, which is expressed in hippocampal cells and in mammary cells. Unless otherwise noted, any reference herein to sel-10 will be understood to refer to human sel-10, and to encompass both hippocampal and mammary sel-10. Fragments of hippocampal sel-10 and mammary sel-10 are also provided.

In a preferred embodiment, the invention provides an isolated nucleic acid molecule comprising a polynucleotide having a sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a human sel-10 polypeptide having the complete amino acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7, or as encoded by the cDNA clone contained in ATCC Deposit No.98978;

(b) a nucleotide sequence encoding a human sel-10 polypeptide having the complete amino acid sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10, or as encoded by the cDNA clone contained in ATCC Deposit No. 98979; and

(c) a nucleotide sequence complementary to the nucleotide sequence of (a) or (b).

In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent conditions to a polynucleotide encoding sel-10, or fragments thereof.

The present invention also provides vectors comprising the isolated nucleic acid molecules of the invention, host cells into which such vectors have been introduced, and recombinant methods of obtaining a sel-10 polypeptide comprising culturing the above-described host cell and isolating the sel-10 polypeptide.

In another aspect, the invention provides isolated sel-10 polypeptides, as well as fragments thereof. In a preferred embodiment, the sel-10 polypeptides have an amino acid sequence selected from the group consisting of SEQ ID NO:3, 4, 5, 6, 7, 8, 9, and 10. Isolated antibodies, both polyclonal and monoclonal, that bind specifically to sel-10 polypeptides are also provided.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A and 1B: Figures 1A and 1B are western blots showing protein expression in HEK293 cells transfected with PS1-C-FLAG, 6-myc-N-sel-10, and APP695NL-KK cDNAs.

Figures 2A and 2B: Figures 2A and 2B are northern blots. Figure 2A is a multiple tissue northern blot probed with the common, mammary *sel-10* mRNA showing ubiquitous expression of the 6.5- and 4.5-Kb transcripts. Figure 2B is a multiple tissue northern blot showing limited expression of the hippocampal *sel-10* mRNA only in brain.

Figures 3A, 3B and 3C. Figures 3A, 3B and 3C are western blots. Figure 3A demonstrates that SEL-10-myc forms a complex with PS1 as shown by immunoprecipitation with anti-PS1 loop antibody. HEK293 cells were transfected with constructs containing SEL-10-myc or PS1, cotransfected with both constructs, transfected with the corresponding vector only control (pcDNA3 or pCS, respectively), or mock transfected without DNA. Cultures were treated with lactacystin (12 μM) to inhibit proteasome function as indicated. The immunoprecipitates were loaded on the left side of the gel and cell lysates on the right. Controls for immunoprecipitation were a nonspecific IgG and just anti-PS1 loop antibody with protein G beads with no cell lysate ("no protein"). The immunoblot was probed with anti-myc antibody to detect SEL-10-myc in the immunoprecipitates and cell lysates. SEL-10-myc is expressed in lysates from cells transfected with SEL-10-myc or cotransfected with SEL-10-myc and PS1, but was detected only as a complex with PS1 in cells cotransfected with the SEL-10-myc and PS1 constructs. Figure 3B demonstrates that the SEL-10-myc/PS1 complex could be detected only when proteasome degradation was inhibited with lactacystin. Figure 3C demonstrates that immunoprecipitation of the SEL-10-myc/PS1 complex with anti-myc antibody indicates that SEL-10-myc associates primarily with full length and high molecular weight forms of PS1. Immunoprecipitates were probed with either anti-PS1 loop or anti-myc antibodies. Longer exposures of the ECL developed Western blot reveal very low levels of PS1-NTF in the immunoprecipitate with SEL-10-myc, but fail to detect PS1-CTF.

Figures 4A and 4B. Figures 4A and 4B are western blots. Figure 4A demonstrates that SEL-10-myc stimulates ubiquitination of PS1. An HEK293 cell line with stable expression of PS1 was transfected with SEL-10-myc. Immunoprecipitation with an anti-PS1 loop antibody was followed by detection with an anti-ubiquitin antibody. Figure 4B demonstrates that increased expression of SEL-10-myc leads to a decrease in PS1-NTF and PS1-CTF with a corresponding increase in the amount of full length PS1. Cell lysates were immunoprecipitated with anti-PS1 loop antibody and then probed on the Western blot with the same antibody to detect PS1 and its processing products.

Figures 5A, 5B and 5C. Figures 5A, 5B and 5C are graphs. Figure 5A demonstrates that transient co-expression of SEL-10-myc with APP increases production of Aβ1-40 and Aβ1-42. The effect is additive with co-expressed PS1. Figure 5B demonstrates that stable expression of SEL-10-myc or PS1 increases endogenous Aβ production. Figure 5C demonstrates that transient expression of APP in the stable cell lines increases exogenous Aβ production over the level seen in a cell line transformed with the pcDNA3.1 vector control.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding human sel-10. The nucleotide sequence of human hippocampal sel-10 (hhsel-10), which sequence is given in SEQ ID NO:1, encodes five hhsel-10 polypeptides (hhsel-10-(1), hhsel-10-(2), hhsel-10-(3), hhsel-10-(4), and hhsel-10-(5), referred to collectively herein as hhsel-10). The nucleotide sequence of human mammary sel-10 (hmsel-10), which sequence is given in SEQ ID NO:2, encodes three hmsel-10 polypeptides (hmSel-10-(1), hmSel-10-(2), and hmsel-10-(3), referred to collectively herein as hmsel-10). The nucleotide sequences of the hhsel-10 polynucleotides are given in SEQ ID NO. 1, where nucleotide residues 45-1928 of SEQ ID NO. 1 correspond to hhsel-10-(1), nucleotide residues 150-1928 of SEQ ID NO. 1 correspond to hhSel-10-(2), nucleotide residues 267-1928 of SEQ ID NO. 1 correspond to hhSel-10-(3), nucleotide residues 291-

1928 of SEQ ID NO. 1 correspond to hhSel-10-(4), and nucleotide residues 306-1928 of SEQ ID NO. 1 correspond to hhSel-10-(5). The nucleotide sequences of the hmSel-10 polynucleotides are given in SEQ ID NO. 2, where nucleotide residues 180-1949 of SEQ ID NO. 2 correspond to hmSel-10-(1), nucleotide residues 270-1949 of SEQ ID NO. 2 correspond to hmSel-10-(2), and nucleotide residues 327-1949 of SEQ ID NO. 2 correspond to hmSel-10-(3). The amino acid sequences of the polypeptides encoded by the hhSel-10 and hm-Sel-10 nucleic acid molecules are given as follows: SEQ ID NOS: 3, 4, 5, 6, and 7 correspond to the hhSel-10-(1), hhSel-10-(2), hhSel-10-(3). hhSel-10-(4), and hhSel-10-(5) polypeptides, respectively, and SEQ ID NOS: 8, 9, and 10 correspond to the hmSel-10-(1), hmSel-10-(2), and hmSel-10-(3) polypeptides, respectively. Unless otherwise noted, any reference herein to sel-10 will be understood to refer to human sel-10, and to encompass all of the hippocampal and mammary sel-10 nucleic acid molecules (in the case of reference to sel-10 nucleic acid, polynucleotide, DNA, RNA, or gene) or polypeptides (in the case of reference to sel-10 protein, polypeptide, amino acid sequnce). Fragments of hippocampal sel-10 and mammary sel-10 nucleic acid molecules and polypeptides are also provided.

The nucleotide sequence of SEQ ID NO:1 was obtained as described in Example 1, and is contained in cDNA clone PNV 102-1, which was deposited on November 9, 1998, at the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110, and given accession number 98978. The nucleotide sequence of SEQ ID NO:2 was obtained as described in Example 1, and is contained in cDNA clone PNV 108-2, which was deposited on November 9, 1998, at the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110, and given accession number 98979.

The human sel-10 polypeptides of the invention share homology with *C. elegans* sel-10, as well as with members of the β-transducin protein family, including yeast CDC4, and human LIS-1. This family is characterized by the presence of an F-box and multiple WD-40 repeats (Li, J., *et al., Proc. Natl. Acad. Sci. U.S.A. 92*:12180-12184 (1995)). The repeats are 20-40 amino acids long and are bounded by gly-his (GH) and trp-asp (WD) residues. The three dimensional structure of β-transducin indicates that the WD40 repeats form the arms of a seven-bladed propeller like structure (Sondek, J., *et al., Nature 379*:369-374 (1996)). Each blade is formed by four alternating pleats of beta-sheet with a pair of the conserved aspartic acid residues in the protein motif forming the limits of one internal beta strand. WD40 repeats are found in over 27 different proteins which represent diverse functional classes (Neer, E.J., *et al., Nature 371*:297-300 (1994)). These regulate cellular functions including cell division, cell fate determination, gene transcription, signal transduction, protein degradation, mRNA modification and vesicle fusion. This diversity in function has led to the hypothesis that β-transducin family members provide a common scaffolding upon which multiprotein complexes can be assembled.

The nucleotide sequence given in SEQ ID NO:1 corresponds to the nucleotide sequence encoding hhsel-10, while the nucleotide sequence given in SEQ ID NO:2 corresponds to the nucleotide sequence encoding hmsel-10. The isolation and sequencing of DNA encoding sel-10 is described below in Examples 1 and 2.

As is described in Examples 1 and 2, automated sequencing methods were used to obtain the nucleotide sequence of sel-10. The sel-10 nucleotide sequences of the present invention were obtained for both DNA strands, and are believed to be 100% accurate. However, as is known in the art, nucleotide sequence obtained by such automated methods may contain some errors. Nucleotide sequences determined by automation are typically at least about 90%, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of a given nucleic acid molecule. The actual sequence may be more precisely determined using manual sequencing methods, which are well known in the art. An error in sequence which results in an insertion or deletion of one or more nucleotides may result in a frame shift in translation such that the predicted amino acid sequence will differ from that which would be predicted from the actual nucleotide sequence of the nucleic acid molecule, starting at the point of the mutation. The sel-10 DNA of the present invention includes cDNA, chemically synthesized DNA, DNA isolated by PCR, genomic DNA, and combinations thereof. Genomic *sel*-10 DNA may be obtained by screening a genomic library with the *sel*-10 cDNA described herein, using methods that are well known in the art. RNA transcribed from *sel*-10 DNA is also encompassed by the present invention.

Due to the degeneracy of the genetic code, two DNA sequences may differ and yet encode identical amino acid sequences. The present invention thus provides isolated nucleic acid molecules having a polynucleotide sequence encoding any of the sel-10 polypeptides of the invention, wherein said polynucleotide sequence encodes a sel-10 polypeptide having the complete amino acid sequence of SEQ ID NOs:3-10, or fragments thereof.

Also provided herein are purified sel-10 polypeptides, both recombinant and non-recombinant. Variants and derivatives of native sel-10 proteins that retain any of the biological activities of sel-10 are also within the scope of the present invention. As is described above, the sel-10 polypeptides of the present invention share homology with yeast CDC4. As CDC4 is known to catalyze ubiquitination of specific cellular proteins (Feldman *et al.*, *Cell 91*:221 (1997)), it may be inferred that sel-10 will also have this activity. Assay procedures for demonstrating such activity are well known, and involve reconstitution of the ubiquitinating system using purified human sel-10 protein together with the yeast proteins Cdc4p, Cdc53p and Skp1p, or their human orthologs, and an E1 enzyme, the E2 enzyme Cdc34p or its human ortholog, ubiquitin, a target protein and an ATP regenerating system (Feldman *et al.*, 1997). Skp1p associates with Cdc4p through a protein domain called an F-box (Bai *et al.*, *Cell 86*:263 (1996)). The F-box protein motif is found in yeast CDC4, *C. elegans* sel-10, mouse sel-10 and human sel-10. The sel-10 ubiquitination system may be reconstituted with the *C. elegans* counterparts of the yeast components, *e.g.*, cul-1 (also known as lin-19) protein substituting for Cdc53p (Kipreos *et al.*, *Cell 85*:829 (1996)) and the protein F46A9 substituting for Skp1p, or with their mammalian counterparts, *e.g.*, Cul-2 protein substituting for Cdc53p (Kipreos *et al.*, 1996) and mammalian Skp1p substituting for yeast Skp1p. A phosphorylation system provided by a protein kinase is also included in the assay system as per Feldman *et al.*, 1997.

Sel-10 variants may be obtained by mutation of native sel-10-encoding nucleotide sequences, for example. A sel-10 variant, as referred to herein, is a polypeptide substantially homologous to a native sel-10 but which has an amino acid sequence different from that of native sel-10 because of one or more deletions, insertions, or substitutions in the amino acid sequence. The variant amino acid or nucleotide sequence is preferably at least about 80% identical, more preferably at least about 90% identical, and most preferably at least about 95% identical, to a native sel-10 sequence. Thus, a variant nucleotide sequence which contains, for example, 5 point mutations for every one hundred nucleotides, as compared to a native sel-10 gene, will be 95% identical to the native protein. The percentage of sequence identity, also termed homology, between a native and a variant sel-10 sequence may also be determined, for example, by comparing the two sequences using any of the computer programs commonly employed for this purpose, such as the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wisconsin), which uses the algorithm of Smith and Waterman (*Adv. Appl. Math. 2:* 482-489 (1981)).

Alterations of the native amino acid sequence may be accomplished by any of a number of known techniques. For example, mutations may be introduced at particular locations by procedures well known to the skilled artisan, such as oligonucleotide-directed mutagenesis, which is described by Walder *et al.* (*Gene 42*:133 (1986)); Bauer *et al.* (*Gene 37*:73 (1985)); Craik (*BioTechniques*, January 1985, pp. 12-19); Smith *et al.* (*Genetic Engineering: Principles and Methods*, Plenum Press (1981)); and U.S. Patent Nos. 4,518,584 and 4,737,462.

Sel-10 variants within the scope of the invention may comprise conservatively substituted sequences, meaning that one or more amino acid residues of a sel-10 polypeptide are replaced by different residues that do not alter the secondary and/or tertiary structure of the sel-10 polypeptide. Such substitutions may include the replacement of an amino acid by a residue having similar physicochemical properties, such as substituting one aliphatic residue (Ile, Val, Leu or Ala) for another, or substitution between basic residues Lys and Arg, acidic residues Glu and Asp, amide residues Gln and Asn, hydroxyl residues Ser and Tyr, or aromatic residues Phe and Tyr. Further information regarding making phenotypically silent amino acid exchanges may be found in Bowie *et al., Science 247*:1306-1310 (1990). Other sel-10 variants which might retain substantially the biological activities of sel-10 are those where amino acid substitutions have been made in areas outside functional regions of the protein.

In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent conditions to a portion of the nucleic acid molecules described above, *e.g.*, to at least about 15 nucleotides, preferably to at least about 20 nucleotides, more preferably to at least about 30 nucleotides, and still more preferably to at least about from 30 to at least about 100 nucleotides, of one of the previously described nucleic acid molecules. Such portions of nucleic acid molecules having the described lengths refer to, *e.g.*, at least about 15 contiguous nucleotides of the reference nucleic acid molecule. By stringent hybridization conditions is intended overnight incubation at about 42/C for about 2.5 hours in 6 X SSC/0.1% SDS, followed by washing of the filters in 1.0 X SSC at 65/C, 0.1% SDS.

Fragments of the sel-10-encoding nucleic acid molecules described herein, as well as polynucleotides capable of hybridizing to such nucleic acid molecules may be used as a probe or as primers in a polymerase chain reaction (PCR). Such probes may be used, *e.g.*, to detect the presence of *sel*-10 nucleic acids in *in vitro* assays, as well as in Southern and northern blots. Cell types expressing sel-10 may also be identified by the use of such probes. Such procedures are well known, and the skilled artisan will be able to choose a probe of a length suitable to the particular application. For PCR, 5' and 3' primers corresponding to the termini of a desired sel-10 nucleic acid molecule are employed to isolate and amplify that sequence using conventional techniques.

Other useful fragments of the sel-10 nucleic acid molecules are antisense or sense oligonucleotides comprising a single-stranded nucleic acid sequence capable of binding to a target sel-10 mRNA (using a sense strand), or sel-10 DNA (using an antisense strand) sequence.

In another aspect, the invention includes sel-10 polypeptides with or without associated native pattern glycosylation. Sel-10 expressed in yeast or mammalian expression systems (discussed below) may be similar to or significantly different from a native sel-10 polypeptide in molecular weight and glycosylation pattern. Expression of sel-10 in bacterial expression systems will provide non-glycosylated sel-10.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. Sel-10 polypeptides may be recovered and purified from recombinant cell cultures by well-known methods, including ammonium sulfate or ethanol precipitation, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. In a preferred embodiment, high performance liquid chromatography (HPLC) is employed for purification.

The present invention also relates to vectors comprising the polynucleotide molecules of the invention, as well as host cell transformed with such vectors. Any of the polynucleotide molecules of the invention may be joined to a vector, which generally includes a selectable marker and an origin of replication, for propagation in a host. Because the invention also provides sel-10 polypeptides expressed from the polynucleotide molecules described above, vectors for the expression of sel-10 are preferred. The vectors include DNA encoding any of the sel-10 polypeptides described above or below, operably linked to suitable transcriptional or translational regulatory sequences, such as those derived from a mammalian, microbial, viral, or insect gene. Examples of regulatory sequences include transcriptional promoters, operators, or enhancers, mRNA ribosomal binding sites, and appropriate sequences which control transcription and translation. Nucleotide sequences are operably linked when the regulatory sequence functionally relates to the DNA encoding sel-10. Thus, a promoter nucleotide sequence is operably linked to a sel-10 DNA sequence if the promoter nucleotide sequence directs the transcription of the sel-10 sequence.

Selection of suitable vectors to be used for the cloning of polynucleotide molecules encoding sel-10, or for the expression of sel-10 polypeptides, will of course depend upon the host cell in which the vector will be transformed, and, where applicable, the host cell from which the sel-10 polypeptide is to be expressed. Suitable host cells for expression of sel-10 polypeptides include prokaryotes, yeast, and higher eukaryotic cells, each of which is discussed below.

The sel-10 polypeptides to be expressed in such host cells may also be fusion proteins which include regions from heterologous proteins. Such regions may be included to allow, *e.g.*, secretion, improved stability, or facilitated purification of the polypeptide. For example, a sequence encoding an appropriate signal peptide can be incorporated into expression vectors. A DNA sequence for a signal peptide (secretory leader) may be fused in-frame to the sel-10 sequence so that sel-10 is translated as a fusion protein comprising the signal peptide. A signal peptide that is functional in the intended host cell promotes extracellular secretion of the sel-10 polypeptide. Preferably, the signal sequence will be cleaved from the sel-10 polypeptide upon secretion of sel-10 from the cell. Non-limiting examples of signal sequences that can be used in practicing the invention include the yeast I-factor and the honeybee melatin leader in sf9 insect cells.

In a preferred embodiment, the sel-10 polypeptide will be a fusion protein which includes a heterologous region used to facilitate purification of the polypeptide. Many of the available peptides used for such a function allow selective binding of the fusion protein to a binding partner. For example, the sel-10 polypeptide may be modified to comprise a peptide to form a fusion protein which specifically binds to a binding partner, or peptide tag. Non-limiting examples of such peptide tags include the 6-His tag, thioredoxin tag, FLAG tag, hemaglutinin tag, GST tag, and OmpA signal sequence tag. As will be understood by one of skill in the art, the binding partner which recognizes and binds to the peptide may be any molecule or compound including metal ions *(e.g.,* metal affinity columns), antibodies, or fragments thereof, and any protein or peptide which binds the peptide. These tags may be recognized by fluorescein or rhodamine labeled antibodies that react specifically with each type of tag

Suitable host cells for expression of sel-10 polypeptides include prokaryotes, yeast, and higher eukaryotic cells. Suitable prokaryotic hosts to be used for the expression of sel-10 include bacteria of the genera *Escherichia, Bacillus, and Salmonella,* as well as members of the genera *Pseudomonas, Streptomyces,* and *Staphylococcus.* For expression in, *e.g., E. coli,* a sel-10 polypeptide may include an N-terminal methionine residue to facilitate expression of the recombinant polypeptide in a prokaryotic host. The N-terminal Met may optionally then be cleaved from the expressed sel-10 polypeptide.

Expression vectors for use in prokaryotic hosts generally comprise one or more phenotypic selectable marker genes. Such genes generally encode, *e.g.*, a protein that confers antibiotic resistance or that supplies an auxotrophic requirement. A wide variety of such vectors are readily available from commercial sources. Examples include pSPORT vectors, pGEM vectors (Promega), pPROEX vectors (LTI, Bethesda, MD), Bluescript vectors (Stratagene), and pQE vectors (Qiagen).

Sel-10 may also be expressed in yeast host cells from genera including *Saccharomyces, Pichia,* and *Kluveromyces.* Preferred yeast hosts are *S. cerevisiae* and *P. pastoris.* Yeast vectors will often contain an origin of replication sequence from a 2T yeast plasmid, an autonomously replicating sequence (ARS), a promoter region, sequences for polyadenylation, sequences for transcription termination, and a selectable marker gene.

Vectors replicable in both yeast and *E. coli* (termed shuttle vectors) may also be used. In addition to the above-mentioned features of yeast vectors, a shuttle vector will also include sequences for replication and selection in *E. coli.* Direct secretion of sel-10 polypeptides expressed in yeast hosts may be accomplished by the inclusion of nucleotide sequence encoding the yeast I-factor leader sequence at the 5' end of the sel-10-encoding nucleotide sequence.

Insect host cell culture systems may also be used for the expression of Sel-10 polypeptides. In a preferred embodiment, the sel-10 polypeptides of the invention are expressed using a baculovirus expression system. Further information regarding the use of baculovirus systems for the expression of heterologous proteins in insect cells are reviewed by Luckow and Summers, *Bio/Technology* 6:47 (1988).

In another preferred embodiment, the sel-10 polypeptide is expressed in mammalian host cells. Non-limiting examples of suitable mammalian cell lines include the COS-7 line of monkey kidney cells (Gluzman *et al., Cell* 23:175 (1981)) and Chinese hamster ovary (CHO) cells.

The choice of a suitable expression vector for expression of the sel-10 polypeptides of the invention will of course depend upon the specific mammalian host cell to be used, and is within the skill of the ordinary artisan. Examples of suitable expression vectors include pcDNA3 (Invitrogen) and pSVL (Pharmacia Biotech). Expression vectors for use in mammalian host cells may include transcriptional and translational control sequences derived from viral genomes. Commonly used promoter sequences and enhancer sequences which may be used in the present invention include, but are not limited to, those derived from human cytomegalovirus (CMV), Adenovirus 2, Polyoma virus, and Simian virus 40 (SV40).

Methods for the construction of mammalian expression vectors are disclosed, for example, in Okayama and Berg (*Mol. Cell. Biol. 3*:280 (1983)); Cosman *et al.* (*Mol. Immunol. 23*:935 (1986)); Cosman *et al.* (*Nature 312*:768 (1984)); EP-A-0367566; and WO 91/18982.

The polypeptides of the present invention may also be used to raise polyclonal and monoclonal antibodies, which are useful in diagnostic assays for detecting sel-10 polypeptide expression. Such antibodies may be prepared by conventional techniques. See, for example, *Antibodies: A Laboratory Manual*, Harlow and Land (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1988); *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Kennet *et al.* (eds.), Plenum Press, New York (1980).

The sel-10 nucleic acid molecules of the present invention are also valuable for chromosome identification, as they can hybridize with a specific location on a human chromosome. There is a current need for identifying particular sites on the chromosome, as few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. The relationship between genes and diseases that have been mapped to the same chromosomal region can then be identified through linkage analysis, wherein the coinheritance of physically adjacent genes is determined. Whether a gene appearing to be related to a particular disease is in fact the cause of the disease can then be determined by comparing the nucleic acid sequence between affected and unaffected individuals.

The sel-10 polypeptides of the invention, and the DNA encoding them, may also be used to further elucidate the biological mechanism of AD, and may ultimately lead to the identification of compounds that can be used to alter such mechanisms. The sel-10 polypeptides of the invention are 47.6% identical and 56.7% similar to *C. elegans* sel-10. As is described above, mutations to *C. elegans sel*-10 are known to suppress mutations to *sel*-12 that result in a loss-of-function for egg laying, and also to suppress certain hypomorphic mutations to *lin*-12. Mutations to *C. elegans sel*-12 can also be rescued by either of the human AD-linked genes PS-1 (42.7% identical to *sel*-12) or PS-2 (43.4% identical to *sel*-12). However, human PS-1 with a familial AD-linked mutant has a reduced ability to rescue *sel*-12 mutants (Levitan, D. *et al., Proc. Natl. Acad. Sci. USA 93*: 14940-14944 (1996)).

This demonstrated interchangeability of human and *C. elegans* genes in the notch signaling pathway makes it reasonable to predict that mutations of human sel-10 will suppress mutations to PS-1 or PS-2 that lead to AD, especially in light of the predicted structure of sel-10. As described above, PS-1 and PS-2 mutations that lead to AD are those which interfere with the proteolytic processing of PS-1 or PS-2. The sel-10 polypeptides of the invention are members of the β-transducin protein family, which includes yeast CDC4, a component of an enzyme which functions in the ubiquitin-dependent protein degradation pathway. Thus, human *sel*-10 may regulate presenilin degradation via the ubiquitin-proteasome pathway. Alternatively, or in addition, human sel-10 may alter presenilin function by targeting for degradation through ubiquitination a modulator of presenilin activity, e.g., a negative regulator. Therefore, mutations to *sel*-10 may reverse the faulty proteolytic processing of PS-1 or PS-2 which occurs as a result of mutation to PS-1 or PS-2 or otherwise increase presenilin function. For the same reason, inhibition of sel-10 activity may also act to reverse PS-1 or PS-2 mutations. Thus, it may be hypothesized that compounds which inhibit either the expression or the activity of the human sel-10 polypeptides of the invention may reverse the effects of mutations to PS-1 or PS-2, and thus be useful for the prevention or treatment of AD.

Thus, *C. elegans* may be used as a genetic system for the identification of agents capable of inhibiting the activity or expression of the human sel-10 polypeptides of the invention. A suitable *C. elegans* strain for use in such assays lacks a gene encoding active *C. elegans* sel-10, and exhibits a loss-of-function for egg-laying resulting from an inactivated *sel-12* gene. Construction of *C. elegans* strains having a loss-of-function for egg-laying due to mutation of *sel-12* may be accomplished using routine methods, as both the sequence of *sel-12* (Genebank accession number U35660) and mutations to *sel-12* resulting in a loss-of-function for egg laying are known (*see* Levitan e*t al., Nature 377*: 351-354 (1995), which describes construction of *C. elegans sel-12(ar171)*). An example of how to make such a strain is also given in Levitan *et al.* (*Nature 377*: 351-354 (1995)). Wild-type *C. elegans sel-10* in the *C. elegans sel-12(ar171)*) , is also mutagenized using routine methods, such as the technique used for sel-12 mutagenesis in Levitan *et al., supra.*

In order to identify compounds inhibiting human sel-10 activity, a DNA vector containing a human sel-10 gene encoding any of the wild-type human sel-10 proteins of the invention is introduced into the above-described *C. elegans* strain. In a preferred embodiment, the heterologous human sel-10 gene is integrated into the *C. elegans* genome.

The gene is then expressed, using techniques described in Levitan *et al.* (*Proc. Natl. Acad. Sci. USA 93*: 14940-14944 (1996)). Test compounds are then administered to this strain in order to determine whether a given agent is capable of inhibiting sel-10 activity so as to suppress mutations to *sel-12* or *lin-12* that result in egg-laying defects. Egg-laying in this strain is then determined, e.g. by the assay described in Levitan *et al.* (*Proc. Natl. Acad. Sci. USA 93*: 14940-14944 (1996)). To confirm that the compound's effect on egg-laying is due to inhibition of sel-10 activity, the action of the compound can be tested in a second biochemical or genetic pathway that is known to be affected by loss-of-function mutations in *sel-10* (*e.g.*, further elevation of lin-12 activity in lin-12(d) hypomorphic strains). Such assays may be performed as described in Sundarem and Greenwald (*Genetics 135*: 765-783 (1993)).

Alternatively, compounds are tested for their ability to inhibit the E3 Ubiquitin Ligating Enzyme. Assays procedures for demonstrating such activity are well known, and involve reconstitution of the ubiquitinating system using purified human sel-10 protein together with the yeast proteins Cdc4p, Cdc53p and Skp1p and an E1 enzyme, the E2 enzyme Cdc34p, ubiquitin, a target protein and an ATP regenerating system (Feldman *et al.*, 1997). The sel-10 ubiquitination system may also be reconstituted with the *C. elegans* counterparts of the yeast components, *e.g.*, cul-1 (also known as lin-19) protein substituting for Cdc53p (Kipreos *et al., Cell 85*:829 (1996)) and the protein F46A9 substituting for Skp1p, or with their mammalian counterparts, *e.g.*, Cul-2 protein substituting for Cdc53p (Kipreos *et al., ibid.*) and mammalian Skp1p substituting for yeast Skp1p. A phosphorylation system provided by a protein kinase is also to be included in the assay system as per Feldman *et al.*, 1997.

Alternatively, cell lines which express human sel-10 due to transformation with a human sel-10 cDNA and which as a consequence have elevated APP processing and formation of $A\beta_{1-40}$ or $A\beta_{1-42}$ may also be used for such assays as in Example 3. Compounds may be tested for their ability to reduce the elevated $A\beta$ processing seen in the sel-10 transformed cell line.

Compounds that rescue the egg-laying defect or that inhibit E3 Ubiquitin Ligating Enzyme are then screened for their ability to cause a reduction in the production of A-beta$_{1-40}$ or A-beta$_{1-42}$ in a human cell line. Test compounds are used to expose IMR-32 or other human cell lines known to produce A-beta$_{1-40}$ or A-beta$_{1-42}$ (Asami-Okada *et al.*, *Biochemistry 34*: 10272-10278 (1995)), or in human cell lines engineered to express human

APP at high levels. In these assays, A-beta$_{1-40}$ or A-beta$_{1-42}$ is measured in cell extracts or after release into the medium by ELISA or other assays which are known in the art (Borchelt *et al., Neuron 17*: 1005-1013 (1996); Citron *et al., Nat. Med. 3*: 67-72 (1997)).

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

EXAMPLES

Example 1: Identification of a human homologue to C. elegans sel-10

Results

Identification of sel-10 in ACEDB: Sel-10 maps between the cloned polymorphisms arP3 and TCPARI just to the left of him-5 [ACEDB entry wm95p536]. Three phage lambda clones have been sequenced across the interval, F53C11, F09F3, and F55B12. *Sel-10* is reported to have homology to yeast *cdc4* [ACEDB entry wm97ab259]. Blast search revealed a single ORF with homology to yeast *cdc4* (CC4_YST) within the interval defined by arP3 and TCPARI corresponding to the GenPep entry F55B12.3. F55B12.3, like yeast *cdc4*, is a member of the β-transducin protein family. This family is characterized by the presence of multiple WD40 repeats [Neer, E.J. *et al., Nature 371*: 297-300 (1994)].

Identification of a human sel-*10 homologue, Incyte 028971:* The GenPep entry F55B12.3 was used to search the LifeSeq, LifeSeq FL and EMBL data bases using tblastn. The search revealed multiple homologies to β-transducin family members including LIS-1 (S36113 and P43035), a gene implicated in Miller-Dieker lissencephaly, a *Xenopus laevis* gene, TRCPXEN (U63921), and a human contig in LifeSeq FL, 028971. Since there also are multiple β-transducin family members within the *C. elegans* genome, these were collected using multiple blast searches and then clustered with the *sel-10* candidate genes. Multiple alignments were performed with the DNAStar program Megalign using the Clustal method. This revealed that LIS-1 clustered with T03F6.F, a different β-transducin family member and thus excluded it as a candidate *sel-10* homologue. TRCPXEN clustered with K10B2.1, a gene which also clusters with F55B12.3 and CC4YST, while Incyte 028971 clustered with *sel-10*. Thus, Incyte 028971 appears to encode the human homologue of *C. elegans sel-10*. Sequence homology between *sel-10* and 028971 is strongest in the region of the protein containing 7 repeats of the WD40 motif. The Incyte 028971 contig contains 44 ESTs from multiple libraries including pancreas, lung, T-lymphocytes, fibroblasts, breast, hippocampus, cardiac muscle, colon, and others.

Public EST: Blastx searches with the DNA sequence 028971 against the TREMBLP dataset identified a single homologous mouse EST (W85144) from the IMAGE Library, Soares mouse embryo NbME13.5-14.5. The blastx alignment of 028971 with W85144 and then with F55B12.3 revealed a change in reading frame in 028971 which probably is due to a sequencing error.

Blastn searches of the EMBL EST database with the 028971 DNA sequence revealed in addition to W85144, three human EST that align with the coding sequence of 028971 and six EST that align with the 3' untranslated region of the 028971 sequence.

Protein Motifs: Two protein motifs were identified in F55B12.3 which are shared with yeast *cdc4*, mouse w85144 and human 028971. These are an F-box in the N-terminal domain and seven β-transducin repeats in the C-terminal domain.

Discussion

The *sel-10* gene encodes a member of the β-transducin protein family. These are characterized by the presence of multiple WD40 repeats [Neer, E.J. *et al., Nature 371*: 297-300 (1994)]. The repeats are 20-40 amino acids long and are bounded by gly-his (GH) and trp-asp (WD) residues. Solution of the three dimensional structure of β-transducin indicates that the WD40 repeats form the arms of a seven-bladed propeller like structure [Sondek, J. *et al., Nature 379*: 369-74 (1996)]. Each blade is formed by four alternating pleats of beta-sheet with a pair of the conserved aspartic acid residues in the protein motif forming the limits of one internal beta strand. WD40 repeats are found in over 27 different proteins which represent diverse functional classes [Neer, E.J. *et al., Nature 371*: 297-300 (1994)]. These regulate cellular functions including cell division, cell fate determination, gene transcription, signal transduction, protein degradation, mRNA modification and vesicle fusion. This diversity in function has led to the hypothesis that β-transducin family members provide a common scaffolding upon which multiprotein complexes can be assembled.

The homology of *sel-10*, 28971 and W85144 to the yeast *cdc4* gene suggests a functional role in the ubiquitin-proteasome pathway for intracellular degradation of protein. Mutations of the yeast *cdc4* gene cause cell cycle arrest by blocking degradation of Sic1, an inhibitor of S-phase cyclin/cdk complexes [King, R.W. *et al., Science 274*: 1652-9 (1996)]. Phosphorylation of Sic1 targets it for destruction through the ubiquitin-proteasome pathway. This pathway consists of three linked enzyme reactions that are catalyzed by multiprotein complexes [Ciechanover, A., *Cell 79*: 13-21 (1994); Ciechanover, A. and A.L. Schwartz, *FASEB J. 8*: 182-91 (1994)]. Initially, the C-terminal glycine of ubiquitin is activated by ATP to form a high energy thiol ester intermediate in a reaction catalyzed by the ubiquitin-activating enzyme, E1. Following activation, an E2 enzyme (ubiquitin conjugating enzyme) transfers ubiquitin from E1 to the protein target. In some cases, E2 acts alone. In others, it acts in concert with an E3 ubiquitin-ligating enzyme which binds the protein substrate and recruits an E2 to catalyze ubiquitination. E2 ubiquitin-conjugating enzymes comprise a fairly conserved gene family, while E3 enzymes are divergent in sequence [Ciechanover, A., *Cell 79*: 13-21 (1994); Ciechanover, A. and A.L. Schwartz, *FASEB J. 8*: 182-91 (1994)].

In yeast, mutation of the E2 ubiquitin-conjugating enzyme, cdc34, causes cell cycle arrest through failure to degrade the Sic1 inhibitor of the S-phase cyclin/cdk complex [King, R.W. *et al., Science 274*: 1652-9 (1996)]. Sic1 normally is degraded as cells enter the G1-S phase transition, but in the absence of cdc34, Sic1 escapes degradation and its accumulation causes cell cycle arrest. Besides cdc34, cdc4 is one of three other proteins required for the G1-S phase transition. The other two are cdc53 and Skp1. As discussed above, *cdc4* contains two structural motifs, seven WD40 repeats (which suggests that the protein forms a beta-propeller) and a structural motif shared with cyclin F which is an interaction domain for Skp1 [Bai, C. *et al., Cell 86*: 263-74 (1996)]. Insect cell lysates containing cdc53, *cdc4* and skp1 (and also ubiquitin, cdc34 and E1) can transfer ubiquitin to Sic1 suggesting that one or more of these components functions as an E3 ubiquitin-ligating enzyme [King, R.W. *et al., Science 274*: 1652-9 (1996)]. Increased expression of either cdc4 or Skp1 partially rescues loss of the other.

In *C. elegans*, mutation of *sel-10* has no visible phenotype indicating that *sel-10* does not play a role in regulation of the cell-cycle. A closely related, *C. elegans* β-transducin family member, K10B2.6 may play that role as it clusters with the gene TRCP_XEN from Xenopus laevis which rescues yeast cell cycle mutants arrested in late anaphase due to a failure to degrade cyclin B [Spevak,W. *et al., Mol. Cell. Biol. 13*: 4953-66 (1993)]. If *sel*-10 does encode a component of an E3-ubiquitin ligating enzyme, how might it suppress *sel-12* and enhance *lin-12* mutations? The simplest hypothesis is that sel-10 regulates degradation of both proteins via the ubiquitin-proteasome pathway. Both sel-12 and lin-12 are transmembrane proteins. Sel-*12* crosses the membrane 8 times such that its N- and C-termini face the cytosol [Kim, T.W. *et al., J. Biol. Chem. 272*: 11006-10 (1997)], while *lin-12* is a type 1 transmembrane protein (Greenwald, I. and G. Seydoux, *Nature 346*: 197-9 (1990)). Both are ubiquitinated, and in the case of human PS2, steady state levels increase in cells treated with an inhibitor of the proteasome, N-acetyl-L-leucinal-L-norleucinal and lactacystin (Li, X. and I. Greenwald, *Neuron. 17*: 1015-21 (1996)). Alternatively, sel-10 may target for degradation of a negative regulator of presenilin function.

The genetic analysis and protein function suggested by homology to *cdc4* implies that drug inhibitors of human *sel-10* may increase steady state levels of human presenilins. This could potentiate activity of the presenilin pathway and provide a means for therapeutic intervention in Alzheimer's disease.

Example 2: ***5' RACE cloning of a human cDNA encoding Sel-10, an extragenic suppressor of presenilin mutations in C. elegans***

Materials and Methods

Oligonucleotide primers for the amplification of the sel-10 coding sequence from *C. elegans* cDNA were prepared based on the sequence of F55B12.3, identified in Example 1 as the coding sequence for *C. elegans sel-10.* The primers prepared were: 5'-CGGGATCCACCATGGATGATGGATCGATGACACC-3' (SEQ ID NO:11) and 5'-GGAATTCCTTAAGGGTATACAGCATCAAAGTCG-3' (SEQ ID NO:12). *C. elegans* mRNA was converted to cDNA using a BRL Superscript II Preamplification kit. The PCR product was digested with restriction enzymes *Bam*HI and *Eco*RI (LTI, Gaithersberg, MD) and cloned into pcDNA3.1 (Invitrogen). Two isolates were sequenced (ABI, Perkin-Elmer Corp).

The sequence of Incyte clone 028971 (encoding a portion of the human homologue of *C. elegans* sel-10), was used to design four antisense oligonucleotide primers: 5'-TCACTTCATGTCCACATCAAAGTCC-3' (SEQ ID NO:13), 5'-GGTAA-TTACAAGTTCTTGTTGAACTG (SEQ ID NO:14), 5'-CCCTGCAACGTGTGT-

AGACAGG-3' (SEQ ID NO:15), and 5'-CCAGTCTCTGCATTCCACACTTTG-3' (SEQ ID NO:16) to amplify the missing 5' end of human *sel*-10. The Incyte LifeSeq "Electronic Northern" analysis was used to identify tissues in which *sel*-10 was expressed. Two of these, hippocampus and mammary gland, were chosen for 5' RACE cloning using a CloneTech Marathon kit and prepared Marathon-ready cDNA from hippocampus and mammary gland. PCR products were cloned into the TA vector pCR3.1 (Invitrogen), and isolates were sequenced. An alternate 5' oligonucleotide primer was also designed based on Incyte clones which have 5' ends that differ from the hippocampal *sel*-10 sequence: 5'-CTCAGACAGGTCAGGACATTTGG-3' (SEQ ID NO:17).

Blastn was used to search Incyte databases LifeSeq and LifeSeqFL. Gap alignments and translations were performed with GCG programs (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wisconsin).

Results

The coding sequence of the C. elegans sel-10: The predicted coding sequence of the *C. elegans sel*-10, F55B12.3, had originally been determined at the Genome Sequencing Center, Washington University, St. Louis, by using the computer program GeneFinder to predict introns and exons in the genomic cosmid F55B12. The hypothetical cDNA sequence was confirmed by amplifying this region from *C. elegans* cDNA, cloning, and sequencing it .

The coding sequence of the human sel-10 gene homologue: All of the 028971 antisense oligonucleotides amplified a 5' RACE product from human hippocampal and mammary cDNA. The longest PCR product from the hippocampal reactions was cloned and sequenced. This PCR reaction was designed to generate products which end at the predicted stop codon. Two isolates contained identical sequence which begins 880 bases before the beginning of the 028971 sequence. This sequence was confirmed by comparison with spanning Incyte cDNA clones. The Incyte clones that spanned the 5' end of the human *sel*-10 homologue were not annotated as F55B12.3, as the homology in this region between the human and *C. elegans* genes is low, and as the overlap between these clones and the annotated clones happened to be too small for them to be clustered in the Incyte database or uncovered by our blasting the Incyte database with the 028971 sequence.

The predicted protein sequences of human sel-10 have 47.6% identity and 56.7% similarity to *C. elegans* sel-10. The N-terminus of the human sel-10 sequence begins with 4 in-frame methionines. In addition to the WD40 repeats described above, the human sequence also contains a region homologous to the CDC4 F-box for binding Skp1, as expected for a sel-10 homologue.

Different human sel-10 mRNAs expressed in mammary and hippocampal tissues:

Several additional human sel-10 ESTs which differ from the hippocampal sequence were identified. These are an exact match, which indicates that the alternative transcript is probably real. Comparison of these sequences with the human hippocampal sel-10 sequence shows divergence prior to the 4th in-frame methionine and then exact sequence match thereafter. An oligonucleotide primer specific for the 5' end of this alternative transcript was found to amplify a product from mammary but not hippocampal cDNA. This indicates either that the human sel-10 transcript undergoes differential splicing in a tissue-specific fashion or that the gene contains multiple, tissue specific promoters.

Discussion

5'RACE and PCR amplification were used to clone a full-length cDNA encoding the human homologue of the *C. elegans* gene, sel-10. Sequence analysis confirms the earlier prediction that sel-10 is a member of the CDC4 family of proteins containing F-Box and WD40 Repeat domains. Two variants of the human sel-10 cDNA were cloned from hippocampus and mammary gland which differed in 5' sequence preceding the apparent site of translation initiation. This implies that the gene may have two or more start sites for transcription initiation which are tissue-specific or that the pattern of exon splicing is tissue-specific.

EXAMPLE 3: Expression Of Epitope-Tagged Sel-10 In Human Cells , and PerturbationOf Amyloid β Peptide Processing By Human Sel-10

Materials And Methods

Construction of Epitope-Tagged Sel-10: Subcloning, Cell Growth and Transfection:

An EcoR1 site was introduced in-frame into the human sel-10 cDNA using a polymerase chain reaction (PCR) primed with the oligonucleotides 237 (5'-GGAATTC-CATGAAAAGATTGGACCATGGTTCTG-3') (SEQ ID NO:18) and 206 (5'-GGA-

ATTCCTCACTTCATGT-CACATCAAAGTCCAG-3') (SEQ ID NO:19). The resulting PCR product was cloned into the EcoR1 site of the vector pCS2+MT. This fused a 5' 6-myc epitope tag in- frame to the fifth methionine of the hippocampal sel-10 cDNA, i.e., upstream of nucleotide 306 of the sequence given in SEQ ID NO:1. The nucleotide sequence of this construct, designated 6myc-N-sel-10, is given in SEQ ID NO: 20, while the amino acid sequence of the polypeptide encoded thereby is given in SEQ ID NO: 21. The hippocampal and mammary sel-10 cDNA diverge upstream of this methionine. A PS1 cDNA with a 3'-FLAG tag (PS1-C-FLAG) was subcloned into the pcDNA3.1 vector. An APP cDNA containing the Swedish NL mutation and an attenuated ER retention sequence consisting of the addition of a di-lysyl motif to the C-terminus of APP695 (APP695NL-KK) was cloned into vector pIRES-EGFP (Mullan et al., Nat Genet 1992 Aug;1(5):345-7). HEK293 and IMR32 cells were grown to 80% confluence in DMEM with 10% FBS and transfected with the above cDNA. A total of 10 mg total DNA/6x10^6 cells was used for transfection with a single plasmid. For cotransfections of multiple plasmids, an equal amount of each plasmid was used for a total of 10 mg DNA using LipofectAmine (BRL).

In order to construct C-term V5 his tagged sel-10 and the C-term mychis tagged sel-10, the coding sequence of human hippocampal sel-10 was amplified using oligonucleotides primers containing a KpnI restriction site on the 5' primer: 5'-GGGTA-CCCCTCATTATTCCCTCGAGTTCTTC-3' (SEQ ID NO:22) and an EcoRI site on the 3' primer: 5'-GGAATTCCTTCATGTCCACATCAAAGTCC-3' (SEQ ID NO:23), using the original human sel-10 RACE pcr product as template. The product was digested with both KpnI and EcoRI and cloned into either the vector pcDNA6/V5-His A or pcDNA3.1/Myc-His(+) A (Invitrogen). The nucleotide sequence of independent isolates was confirmed by dideoxy sequencing. The nucleotide sequence of the C-term V5 his tagged sel-10 is given in SEQ ID NO: 24, while the amino acid sequence of the polypeptide encoded thereby is given in SEQ ID NO: 25. The nucleotide sequence of independent isolates was confirmed by dideoxy sequencing. The nucleotide sequence of the C-term mychis tagged sel-10 is given in SEQ ID NO: 26, while the amino acid sequence of the polypeptide encoded thereby is given in SEQ ID NO: 27.

Clonal Selection of transformed cells by FACS: Cell samples were analyzed on an EPICS Elite ESP flow cytometer (Coulter, Hialeah, FL) equipped with a 488 nm excitation line supplied by an air-cooled argon laser. EGFP emission was measured through a 525 nm band-pass filter and fluorescence intensity was displayed on a 4-decade log scale after gating on viable cells as determined by forward and right angle light scatter. Single green cells were separated into each well of one 96 well plate containing growth medium without G418. After a four day recovery period, G418 was added to the medium to a final concentration of 400 mg/ml. Wells with clones were expanded from the 96 well plate to a 24 well plate and then to a 6 well plate with the fastest growing colonies chosen for expansion at each passage.

Immunofluorescence: Cells grown on slides were fixed 48 hrs after transfection with 4% formaldehyde and 0.1% Triton X-100 in PBS for 30 min on ice and blocked with 10% Goat serum in PBS (blocking solution) 1 hr RT (i.e., 25°C), followed by incubation with mouse anti-myc (10 mg/ml) or rabbit anti-FLAG (0.5 mg/ml) antibody 4°C O/N and then fluorescein-labeled goat anti-mouse or anti-rabbit antibody (5mg/ml) in blocking solution 1 hr at 25°C.

Western blotting: Cell lysates were made 48 hrs after transfection by incubating 10^5 cells with 100 ml TENT (50 mM Tris-HCl pH 8.0, 2 mM EDTA, 150 mM NaCl, 1% Triton X-100, 1x protease inhibitor cocktail) 10 min on ice followed by centrifugation at 14,000 g. The supernatant was loaded on 4-12% NuPage gels (50 mg protein/lane) and electrophoresis and transfer were conducted using an Xcell II Mini-Cell system (Novex). The blot was blocked with 5% milk in PBS 1 hr RT and incubated with anti-myc or anti-FLAG antibody (described in "Immunofluorescence" above) 4°C O/N, then sheep anti-mouse or anti-rabbit antibody-HRP (0.1 mg/ml) 1 hr RT, followed by Supersignal (Pierce) detection.

ELISA: Cell culture supernatant or cell lysates (100 ml formic acid/10^6 cells) were assayed in the following double antibody sandwich ELISA, which is capable of detecting levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ peptide in culture supernatant.

Human Aβ 1-40 or 1-42 was measured using monoclonal antibody (mAb) 6E10 (Senetek, St. Louis, MO) and biotinylated rabbit antiserum 162 or 164 (NYS Institute for Basic Research, Staten Island, NY) in a double antibody sandwich ELISA. The capture antibody 6E10 is specific to an epitope present on the N-terminal amino acid residues 1-16 of hAβ. The conjugated detecting antibodies 162 and 164 are specific for hAβ 1-40 and 1-42, respectively. The sandwich ELISA was performed according to the method of Pirttila *et al.* (*Neurobiology of Aging 18:* 121-7 (1997)). Briefly, a Nunc Maxisorp 96 well immunoplate was coated with 100µl/well of mAb 6E10 (5µg/ml) diluted in 0.1M carbonate-bicarbonate buffer, pH 9.6 and incubated at 4°C overnight. After washing the plate 3x with 0.01M DPBS (Modified Dulbecco's Phosphate Buffered Saline (0.008M sodium phosphate, 0.002M potassium phosphate, 0.14M sodium chloride, 0.01 M potassium chloride, pH 7.4) from Pierce, Rockford, Il) containing 0.05% of Tween-20 (DPBST), the plate was blocked for 60 min with 200μl of 10% normal sheep serum (Sigma) in 0.01M DPBS to avoid non-specific binding. Human Aβ 1-40 or 1-42 standards 100μl/well (Bachem, Torrance, CA) diluted, from a 1mg/ml stock solution in DMSO, in non transfected conditioned cell medium was added after washing the plate, as well as 100μl/well of sample i.e. filtered conditioned medium of transfected cells. The plate was incubated for 2 hours at room temperature and 4°C overnight. The next day, after washing the plate, 100μl/well biotinylated rabbit antiserum 162 1:400 or 164 1:50 diluted in DPBST + 0.5% BSA was added and incubated at room temperature for 1hr 15 min. Following washes, 100μl/well neutravidin-horseradish peroxidase (Pierce, Rockford, Il) diluted 1:10,000 in DPBST was applied and incubated for 1 hr at room temperature. After the last washes 100μl/well of o-phenylnediamine dihydrochloride (Sigma Chemicals, St. Louis, MO) in 50mM citric acid/100mM sodium phosphate buffer (Sigma Chemicals, St. Louis, MO), pH 5.0, was added as substrate and the color development was monitored at 450nm in a kinetic microplate reader for 20 min. using Soft max Pro software.

Results

Transfection of HEK293 cells: Transfection efficiency was monitored through the use of vectors that express green fluorescent protein (GFP) or by immunofluorescent detection of epitope-tagged sel-10 or PS1. An N-terminal 6-myc epitope was used to tag human sel-10 (6myc-N-sel-10), while PS1 was tagged with a C-terminal FLAG epitope (PS1-C-FLAG). APP695 was modified by inclusion of the Swedish NL mutation to increase Aβ processing and an attenuated endoplasmic reticulum (ER) retention signal consisting of a C-terminal di-lysine motif (APP695NL-KK). The di-lysine motif increases Aβ processing about two fold. The APP695NL-KK construct was inserted into the first cistron of a bicistronic vector containing GFP (pIRES-EGFP, Invitrogen) to allow us to monitor transfection efficiency. Transfection efficiency in HEK293 cells was about 50% for transfections with a single plasmid DNA. For cotransfections with two plasmids, about 30-40% of the cells expressed both proteins as detected by double immunofluorescence.

Expression of recombinant protein in transfected HEK293 cells was confirmed by Western blot as illustrated for PS1-C-FLAG and 6myc-N-sel-10 (Fig 1A). In the case of cotransfections with three plasmids (PS1-C-FLAG + 6myc-N-sel-10 + APP), all three proteins were detected in the same cell lysate by Western blot (Figure 1B) using appropriate antibodies.

Effect of 6myc-N-sel-10 and PS1-C-FLAG on Aβ processing: Cotransfection of APP695NL-KK with 6myc-N-sel-10 or PS1-C-FLAG into HEK293 cells increased the release of Ab1-40 and Ab1-42 peptide into the culture supernatant by 2- to 3-fold over transfections with just APP695NL-KK (Table 1). Cotransfection of APP695NL-KK with both 6myc-N-sel-10 and PS1-C-FLAG increased Ab release still further (i.e., 4- to 6-fold increase). In contrast, the ratio of Ab1-42/ (Ab1-40 + Ab1-42) released into the supernatant decreased about 50%. The subtle decrease in the ratio of Ab1-42/ (Ab1-40 + Ab1-42) reflects the larger increase in Ab 1-40 relative to Ab 1-42. Neither 6myc-N-sel-10 nor PS1-C-FLAG affected endogenous Ab production in HEK293 cells. Similar observations were also obtained in IMR32 cells (Table 2). However, IMR32 cells transfected less well than HEK293 cells, so the stimulation of APP695NL-KK processing by cotransfection with 6myc-N-sel-10 or PS1-C-FLAG was lower.

Levels of Ab 1-40 expressed in HEK293 cells transfected with APP695NL-KK were sufficient to measure Ab peptide in both the culture supernatant and cell pellet. Considerably more Ab 1-40 is detected in the HEK293 cell pellet than in the supernatant in cells transfected with just APP695NL-KK. Cotransfection with 6myc-N-sel-10 or PS1-C-FLAG proportionately decreased Ab 1-40 in the cell pellet and increased Ab in the culture supernatant. This implies that 6myc-N-sel-10 and PS1-C-FLAG alter processing or trafficking of APP such that proportionately more Ab is released from the cell.

Effect of 6myc-N-sel-10 and PS1-C-FLAG expression on endogenous Aβ processing: The effect of 6myc-N-sel-10 on the processing of endogenous APP by human cells was assessed by creating stably transformed HEK293 cell lines expressing these proteins. Two cell lines expressing 6myc-N-sel-10 were derived (sel-10/2 & sel-10/6) as well as a control cell line transformed with pcDNA3.1 vector DNA. Both 6myc-N-sel-10 cell lines expressed the protein as shown by Western blot analysis. Endogenous production of Ab 1-40 was increased in both 6myc-N-sel-10 cell lines in contrast to vector DNA transformed cells Table 3). In addition, stable expression of 6myc-N-sel-10 significantly increased Ab production after transfection with APP695NL-KK plasmid DNA (Table 3). Similar results were obtained with 6 stable cell lines expressing PS1-C-FLAG. All 6 cell lines showed significant elevation of endogenous Aβ processing and all also showed enhanced processing of Ab after transfection with APP695NL-KK (Table 3). In addition, the increase of Aβ processing seen with 6myc-N-sel-10 was also seen with sel-10 tagged at the C-terminus with either mychis or v5his (See Table 4). Both C-terminal and N-terminal tags resulted in an increase in Aβ processing.

Discussion

These data suggest that , when over expressed, 6myc-N-sel-10 as well as PS1-C-FLAG alter Aβ processing in both transient and stable expression systems. A 6-myc epitope tag was used in these experiments to allow detection of sel-10 protein expression by Western blot analysis. If as its sequence homology to yeast CDC4 suggests, sel-10 is an E2-E3 ubiquitin ligase, it should be possible to identify the proteins it targets for ubiquitination. Since the presenilins are degraded via the ubiquitin-proteasome pathway, PS1 & PS2 are logical targets of sel-10 catalyzed ubiquitination [Kim *et al., J. Biol. Chem. 272*:11006-11010 (1997)]. How sel-10 affects Aβ processing is not understood at this point. In the future, it will be necessary to determine if sel-10 & PS1 increase Aβ processing by altering production, processing, transport, or turn-over of APP, and whether the effect of PS1 is mediated or regulated by sel-10.

These experiments suggest that sel-10 is a potential drug target for decreasing Ab levels in the treatment of AD. They also show that *C. elegans* is an excellent model system in which to investigate presenilin biology in the context of AD. Thus, as is shown by cotransfection experiments, as well as in stable transformants, expression of 6myc-N-sel10 or PS1-C-FLAG increases Aβ processing. An increase in Aβ processing was seen in both HEK293 cells and IMR32 cells after cotransfection of 6myc-N-sel10 or PS1-C-FLAG with APP695NL-KK. In stable transformants of HEK293 cells expressing 6myc-Sel10 or PS1-C-FLAG, an increase in endogenous Aβ processing was observed, as well as an increase in Aβ processing after transfection with APP695NL-KK. This suggests that inhibitors of either sel-10 and/or PS1, may decrease Aβ processing, and could have therappeutic potential for Alzheimer's disease.

***Example 4*: SEL-10 Interacts with Presenilin 1, Facilitates Its Ubiquitination, and Alters Aβ Production**

Mutations in the presenilin genes (PS1 or PS2) in man cause autosomal dominant early onset Alzheimer's disease. These have been linked to alterations in the processing of the amyloid protein precursor (APP) [1,2]. The presenilins are membrane proteins with 6-8 transmembrane domains [3,4], which localize to the endoplasmic reticulum, Golgi complex, nuclear envelope, kinetochore and centrosome [5,6]. Mutations in *sel-12*, a Caenorhabditis elegans presenilin cause a defect in egg laying[7]. *Sel-12* is one of three nematode presenilin homologous (*sel-12*, *hop-1* and *spe-4*) [7-9]. The *sel-12* mutant phenotype can be rescued by human PS1 or PS2 [10], indicating that PS1, PS2 and *sel-12* are functional homologues. Mutations in *sel-12* cause a defect in egg laying by altering signaling through the Notch/*lin-12* pathway. The *sel-12* mutant phenotype can be suppressed by loss of function mutations in a second gene, *sel-10* [11], which probably results in rescue of the egg laying defect by increasing the activity of a functionally redundant presenilin, *hop-1* [8,9]. SEL-10 is a homologue of yeast Cdc4, a member of the SCF (Skp1-Cdc53/CUL1-F-box protein) E2-E3 ubiquitin ligase family [12]. In this study, we show that human SEL-10 interacts with PS1 and enhances PS1 ubiquitination, thus altering cellular levels of unprocessed PS1 and its N- and C-terminal fragments. This leads to an alteration in the metabolism of APP and to an increase in the production of amyloid β-peptide, the principal component of amyloid plaque in Alzheimer's disease [13].

The SCF E2-E3 ubiquitin ligases contain a catalytic core consisting of Skp1, Rbx1, Cdc53/CUL-1 and an E2 ubiquitin transferase, Cdc34 [14]. These are targeted to substrates for ubiquitination by adapter proteins (e.g., Cdc4, Grr1, Met30, β-TrCP) containing an F-box motif and WD40 repeats [15]. There is evidence that presenilins are ubiquitinated and undergo degradation through the ubiquitin-proteasome pathway [16,17]. Physical interaction between *C. elegans* SEL-10 and SEL-12 has been shown previously [11]. Thus, SEL-10 may be the F-box adaptor protein that recruits presenilins for ubiquitination and subsequent degradation.

Both human and mouse orthologs of C. elegans *sel-10* have been identified in EST databases, although the sequence information is incomplete [12]. We used rapid amplification of cDNA ends (RACE) to clone amplification products containing the full coding sequences for human and mouse *sel-10*. There are two variants of human *sel-10* cDNA from hippocampus (hippocampus form) and mammary gland (common form) which differ in 5' sequence upstream of a common translation initiation site. This may indicate that the human *sel-10* transcript undergoes differential splicing in a tissue-specific fashion or that the gene contains multiple, tissue specific promoters. The hippocampal form contains four in-frame methionines upstream of the common initiation site and the mammary form contains three. Whether or not these encode proteins with different N-termini is not known. The common transcript is ubiquitously expressed in all tissues tested (Fig 2A), while the hippocampal form is present only in brain (Fig 2B). The human *sel-10* gene was localized to chromosome 4q31.2-31.3 by in situ hybridization (data not shown). The predicted protein sequences of human and *C. elegans* SEL-10 have 47.6% amino acid identity and 56.7% similarity. Human SEL-10 contains an F-box domain as found in other SCF family members. It also contains seven WD40/β-transducin repeats [18] as seen in yeast Cdc4p and *C. elegans* SEL-10, suggesting that the protein forms a seven-bladed propeller structure [19].

We first assessed the physical interaction of human SEL-10 and PS1. SEL-10 tagged with an N-terminal 6-Myc epitope and PS1 were transiently co-expressed in human embryonic kidney cells (HEK293) and their interaction was assessed by immuno-precipitation. Complexes between SEL-10 and PS1 could only be detected in the presence of a proteasome inhibitor, lactacystin, which was added to the cultures at the time of transfection. When immunoprecipitated with anti-PS1 loop antibody, only the immuno-preciptate from cotransfected cells contained SEL-10-myc (Fig 3A), indicating that SEL-10 can interact with PS1. Since co-immunoprecipitation was not observed in cells without lactacystin treatment (Fig 3B), this suggests that the complex can only be captured by blocking the entry of PS1 into the proteasome degradation pathway. The interaction between SEL-10 and PS1 was confirmed in the reverse experiment by using anti-myc antibody for immunoprecipitation of SEL-10 (Fig 3C). PS1 is cleaved within the cytoplasmic loop between transmembrane domains six and seven by an unknown protease which generates N- and C-terminal fragments (PS1-NTF and PS1-CTF, respectively). The SEL-10-myc immunoprecipitates contained primarily full length and high molecular weight forms of PS1, but only very low or undetectable amounts of PS1-NTF and no PS1-CTF. This suggests that SEL-10 may bind primarily to unprocessed PS1.

Next, we examined the effect of SEL-10 co-transfection on PS1 ubiquitination. PS1 immunoprecipitated from cotransfected cells contains a higher level of ubiquitination compared to cells transfected with PS1 alone as shown by probing with anti-ubiquitin antibody (Fig 4A). This result demonstrates that complex formation between SEL-10 and PS1 facilitates ubiquitination as implied previously by the need for lactacystin to demonstrate SEL-10/PS1 complex accumulation.

We then investigated how ubiquitination affects PS1 protein level. HEK293 cells were cotransfected with either SEL-10 or PS1 alone or in combination and immunoprecipitates were probed with anti-PS1 loop antibody (Fig. 4B). In cells cotransfected with PS1 and SEL-10 in comparison to cells transfected with PS1 alone, PS1-NTF and PS1-CTF were decreased as were the high molecular weight forms of PS1. However, the amount of unprocessed PS1 appeared to be slightly increased. There are several possible interpretations of this complex cellular effect. The first is that SEL-10 mediated ubiquitination of PS1-CTF and PS1-NTF leads to their degradation. However, the fragments of PS1 are not immunoprecipitated with SEL-10 suggesting that SEL-10 does not directly facilitate ubiquitination of PS1-NTF and PS1-CTF. The slight increase in unprocessed PS1 seen in cells cotransfected with SEL-10 may suggest instead that SEL-10 binding to PS1 or SEL-10 mediated ubiquitination of PS1 inhibits proteolytic processing of PS1-NTF and PS1-CTF leading to accumulation of the unprocessed precursor.

In order to examine the impact of SEL-10 mediated ubiquitination of PS1 on amyloid β-peptide (Aβ) production, SEL-10 was expressed by either transient or stable transfection in HEK293 cells with or without wild-type PS1. Aβ peptide production was measured by enzyme immunoassays that could distinguish the 1-40 and 1-42 forms of the peptide. In transient expression experiments (Fig. 5A), coexpression of SEL-10 with APP increased production and release of Aβ1-40 and Aβ1-42 by more than 2-fold in comparison to APP expression alone. Transient coexpression of PS1 with APP also increased Aβ1-40 and Aβ1-42 levels by more than 3-fold, similar to previous reports [20] although this is not a consistent finding [2]. Coexpression of SEL-10 and PS1 with APP had an additive effect with an increase in Aβ production of approximately 7-fold. No effect on the ratio of Aβ1-42/total Aβ was observed with all values falling in the range 8.5% to 13.5%. The stimulation of Aβ processing due to expression of SEL-10 was consistently observed across experiments, however, the degree of stimulation did vary. To confirm and extend the result, a series of HEK293 cell lines were derived with stable expression of SEL-10 or PS1 (Fig. 5B). The two SEL-10 cell lines obtained showed 4- and 7-fold increases in endogenous Aβ1-40 peptide production compared to HEK293 cells transfected with just the pcDNA3.1 vector. Similarly, six cell lines with stable expression of PS1 showed a 2- to 6-fold increase in endogenous Aβ1-40 peptide production. Transient transfection of the stable cell lines with APP cDNA was performed to confirm and extend this result (Fig. 5C). Production of Aβ was greater in the SEL-10 and PS1 cell lines in comparison to a control HEK293 cell line that had been transformed with just the pcDNA3.1 vector. Since SEL-10 decreases the cellular level of PS1-NTF and PS1-CTF fragments while slightly increasing unprocessed PS1, the increase in unprocessed PS1 may be associated with the increase in Aβ peptide processing observed. Transient expression of PS1 has a similar effect. It increases the cellular level of unprocessed PS1 while having relatively little effect on the levels of PS1-NTF and PS1-CTF fragments.

Our data indicate that SEL-10 interacts with PS1, stimulates PS1 ubiquitination, and recruits it into the proteasome pathway for protein degradation. SEL-10 is likely to function as an adaptor protein that assembles the core catalytic complex of an SCF E2-E3 ubiquitin ligase [14]. Recognition of most SCF substrates by F-box/WD40 repeat adaptor proteins is phosphorylation dependent [15], suggesting that this may be an additional level of cellular regulation of presenilin levels. To demonstrate complex formation between human SEL-10 and PS1 in HEK293 cells required inhibition of proteasome function by lactacystin. In contrast, the C. elegans SEL-10/SEL-12 complex accumulates in human HEK293 cells in the absence of proteasome inhibitors suggesting that nematode SEL-10 is unable to assemble the human core catalytic complex [11]. Degradation of an eight-pass transmembrane protein such as presenilin presents a topological hurdle since the presenilin protein must be extracted from the membrane and delivered to the proteasome. Like presenilins, a number of other multipass integral membrane proteins with large cytoplasmic domains such as the cystic fibrosis transmembrane conductance regulator (CFTR) are degraded through the ubiquitin-proteasome pathway [21, 22]. This pathway also is important for quality control within the endoplasmic reticulum [23] and conceivably could impact on intracellular production of Aβ peptide.

Transfection of PS1 into human cells generally is observed to increase cellular levels of unprocessed PS1, but causes little change in levels of PS1-CTF and PS1-NTF suggesting that processing is under tight cellular control [17], perhaps due to their involvement in apoptotic pathways [24]. As a consequence of increased transient or stable expression of PS1, we see an increase in Aβ peptide production that probably is due to the accumulation of unprocessed PS1. A similar effect on Aβ peptide production and accumulation of unprocessed PS1 is caused by increased expression of SEL-10. SEL-10 may directly regulate PS1 processing by binding to the cleavage site in the large cytoplasmic loop of PS1, or SEL-10 mediated ubiquitination may block PS1 processing. Similarly, a number of PS1 mutations associated with familial Alzheimer's disease have been shown to decrease processing of PS1 into N- and C-terminal fragments, although that is not a consistent finding [25]. However, mutant forms of PS1 are associated with a shift in the ratio of Aβ1-42 and

Aβ1-40, whereas expression of wild-type PS1 or SEL-10 has no effect on the ratio of Aβ1-42/total Aβ [1,2].

The genetic data indicates that SEL-10 is a negative regulator of presenilin activity in *C. elegans.* Loss of SEL-10 function presumably rescues the egg laying defect in *sel-12* mutant worms through facilitation of HOP-1 presenilin activity, perhaps by allowing the increased accumulation of processed N- and C-terminal fragments of HOP-1. *Sel-10* was identified in a screen for mutations that increase presenilin activity [11]. In principle, genetic screens in model organisms such as *C. elegans* or *Drosophila* can be used to find mutations that decrease presenilin activity, the desired therapeutic goal in Alzheimer's disease [26]. Such screens have the potential to identify novel therapeutic targets for this devastating disease.

Methods

Cloning. Incyte clone (028971) was identified as the human homologue of C. elegans *sel-10* and its sequence was used to design four antisense oligonucleotide primers 5'-TCACT-TCATGTCCACATCAAAGTCC-3' (SEQ ID NO: __), 5'-GGTAATTACAAAGTTCTTG-TTGAACTG-3' (SEQ ID NO: __), 5'-CCCTGCAACGTGTGTAGACAGG-3' (SEQ ID NO: __), and 5'-CCAGTCTCTGCATTCCACACTTTG-3' (SEQ ID NO: __), to amplify the remainder of the human *sel-10* sequence. "Electronic Northern" analysis revealed expression of *sel-10* in hippocampus and mammary gland so these tissues were chosen for 5'RACE cloning using Marathon kit (CloneTech). Marathon-ready cDNA from hippocampus and mammary gland were prepared as directed in the kit. PCR products were cloned into the TA vector pCR3.1 (Invitrogen), and isolates were sequenced. An alternate 5' oligonucleotide primer was also designed based on Incyte clones that have 5' ends that differ from the hippocampal sel-10 sequence (5'-CTCAGACAGGTCAGGACATTTGG-3' (SEQ ID NO: __). Blastn was used to search the Incyte databases LifeSeq and LifeSeqFL. Gap alignments and translations were performed with GCG programs.

Plasmids and transfections. The human *sel-10* cDNA was inserted into the EcoR1 site of the vector pCS2+MT (gift of Jan Kitajewski, Columbia University College of Physicians and Surgeons). This fused a 5' 6-myc epitope tag in- frame to the fifth methionine of the hippocampal *sel-10* cDNA. The hippocampal and mammary *sel-10* cDNA diverge upstream of this methionine. A PS1 cDNA with a 3'-FLAG tag (PS1-C-FLAG) was inserted into the pcDNA3.1 vector. An APP cDNA containing the Swedish KM →NL mutation and an attenuated ER retention sequence consisting of a C-terminal di-lysine motif (APP695Sw-KK) was inserted into the pIRES-EGFP vector (Clontech). HEK293 cells were grown to 80% confluence in DMEM with 10% FBS and transfected with the above cDNAs. A total of 10 μg DNA/6 x 10^6 cells was used for transfection with a single plasmid. For cotransfections of multiple plasmids, an equal amount of each plasmid was used for a total of 10 μg DNA using LipofectAmine (BRL). Single cells were sorted into each well of one 96 well plate containing growth medium without G418 by FACS using an EPICS Elite ESP flow cytometer (Coulter, Hialeah, FL) equipped with a 488 nm excitation line supplied by an air-cooled argon laser. After a four day recovery period, G418 was added to the medium to a final concentration of 400 μg/ml for the selection of stably transfected cell lines. Stable expression of a cDNA was confirmed by detection of the specific protein or sequence tag by Western blot.

Immunoprecipitation, Western blot, and ELISA. Aβ and PS1 antibodies have been characterized [6, 27]. Cell lysates with equal amount of protein were precipitated with antibody and protein G-Sepharose at 4 °C for 2 hr and beads were washed three times with TENT buffer [11]. Immunoprecipitates were analyzed by Western blot using 4-12% NuPage Bis-Tris gel and PVDF membrane (Novex), peroxidase-conjugated secondary antibody (Vector) and SuperSignal West Pico Luminol/Enhancer (Pierce). The ELISA for Aβ 1-40 and Aβ 1-42 was performed as described [27].

References:

1. Borchelt, D.R. et al. Familial Alzheimer's disease-linked presenilin 1 variants elevate Abeta1-42/1-40 ratio in vitro and in vivo. Neuron 17, 1005-1013 (1996).

2. Citron, M. et al. Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid beta-protein in both transfected cells and transgenic mice. Nat. Med. 3, 67-72 (1997).

3. Doan, A. et al. Protein topology of presenilin 1. Neuron. 17, 1023-1030 (1996).

4. Li, X. & Greenwald, I. Membrane topology of the C. elegans SEL-12 presenilin. Neuron 17, 1015-1021 (1996).

5. De Strooper, B. et al. Phosphorylation, subcellular localization, and membrane orientation of the Alzheimer's disease-associated presenilins. J. Biol. Chem. 272, 3590-3598 (1997).

6. Li, J. et al., Alzheimer presenilins in the nuclear membrane, interphase kinetochores, and centrosomes suggest a role in chromosome segregation. Cell 90, 917-927 (1997).

7. Levitan, D. & Greenwald, I. Facilitation of lin, 12, mediated signalling by sel, 12, a Caenorhabditis elegans S182 Alzheimer's disease gene. Nature 377, 351- 4 (1995).

8. Li, X. & Greenwald, I. HOP, 1, a Caenorhabditis elegans presenilin, appears to be functionally redundant with SEL, 12 presenilin and to facilitate LIN, 12 and GLP, 1 signaling. Proc. Natl. Acad. Sci. U. S. A. 94,12204- 9 (1997).

9. Westlund, B. et al. Reverse genetic analysis of Caenorhabditis elegans presenilins reveals redundant but unequal roles for sel-12 and hop-1 in Notch-pathway signaling. Proc. Natl. Acad. Sci. U. S. A. 96, 2497-2502 (1999).

10. Levitan, D. et al. Assessment of normal and mutant human presenilin function in Caenorhabditis elegans. Proc. Natl. Acad. Sci. U. S. A. 93, 14940-1494 (1996).

11. Wu, G. et al. Evidence for functional and physical association between Caenorhabditis elegans SEL, 10, a Cdc4p, related protein, and SEL, 12 presenilin. Proc. Natl. Acad. Sci. U. S. A. 95, 15787-1 (1998).

12. Hubbard, E. J. et al. Sel, 10, a negative regulator of lin, 12 activity in Caenorhabditis elegans, encodes a member of the CDC4 family of proteins. Genes. Dev. 11, 3182-93 (1997).

13. Selkoe, D.J. Physiological production of the b-amyloid protein and the mechanism of Alzheimer's disease. Trends Neurosci. 16, 403-409 (1993).

14. Tyers, M. & Willems, A.R. One ring to rule a superfamily of E3 ubiquitin ligases. Science 284, 601-604 (1999).

15. Patton, E.E. et al., Combinatorial control in ubiquitin-dependent proteolysis: don't Skp the F-box hypothesis. Trends Genet. 14, 236-243 (1998).

16. Steiner, H. et al. Expression of Alzheimer's disease-associated presenilin-1 is controlled by proteolytic degradation and complex formation. J. Biol. Chem. 273, 32322-32331 (1998).

17. Kim, T. W. et al. Endoproteolytic cleavage and proteasomal degradation of presenilin 2 in transfected cells. J. Biol. Chem. 272, 11006-11010 (1997).

18. Neer E. J. et al. The ancient regulatory-protein family of WD-repeat proteins. Nature 371, 297-300, (1994).

19. Sondek, J. et al. Crystal structure of a G-protein beta gamma dimmer at 2.1A resolution. Nature 379, 369-374 (1996).

20. Ancolio, K. et al. Alpha-secretase-derived product of beta-amyloid precursor protein is decreased by presenilin 1 mutations linked to familial Alzheimer's disease. J. Neurochem. 69, 2494-2499 (1997).

21. Ward, C. L. et al. Degradation of CFTR by the ubiquitin-proteasome pathway. Cell 83, 121-127 (1995).

22. Johnston, J.A. et al. Aggresomes: a cellular response to misfolded proteins. J. Cell Biol. 143: 1883-1898 (1998).

23. Kopito, R. R. ER quality control: the cytoplasmic connection. Cell. 88, 427-430 (1997).

24. Zhang, Z. et al. Destabilization of beta-catenin by mutations in presenilin-1 potentiates neuronal apoptosis. Nature. 395: 698-702 (1998).

25. Shirotani, K. et al. Effects of presenilin N-terminal fragments on production of amyloid b peptide and accumulation of endogenous presenilins. Neurosci. Let. 262, 37-40 (1999).

26. De Strooper, B. et al. Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. Nature 391, 387-390 (1998).

27. Mehta, P.D. et al. Increased plasma amyloid beta protein 1-42 levels in Down syndrome. Neurosci Lett 241, 13-16 (1998).

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the invention.

The entire disclosure of all publications cited herein are hereby incorporated by reference.

Table 1. Effect of 6myc-N-sel-10 and PS1-C-FLAG transient transfection on Ab levels in HEK293 cell supernatants.

Plasmids Transfected	Ab1-42 ng/ml	Ab1-40 ng/ml	Ab1-42/total Ab ng/ml
pcDNA3	81 ±20	231 ±50	0.26 ±0.05
6myc-N-sel-10	67 ±7	246 ±34	0.21 ±0.03
PS1-C-FLAG	75 ±18	227 ±45	0.25 ±0.03
PS1-C-FLAG + 6myc-N-sel-10	77 ±21	220 ±26	0.25 ±0.03
APP695NL-KK	141 ±27	896 ±103	0.14 ±0.02
APP695NL-KK + 6myc-N-sel-10	308 ±17	2576 ±190	0.11 ±0.00
APP695NL-KK + PS1-C-FLAG	364 ±39	3334 ±337	0.09 ±0.00
APP695NL-KK + PS1-C-FLAG + 6myc-N-sel-10	550 ±20	5897 ±388	0.09 ±0.00

Table 2. Effect of 6myc-N-sel-10 and PS1-C-FLAG transient transfection on Ab levels in IMR32 cell supernatants.

Plasmids Transfected	Ab1-42 ng/ml	Ab1-40 ng/ml	Ab1-42/total Ab ng/ml
pcDNA3	65 ±3	319 ±146	0.19 ±0.06
6myc-N-sel-10	63 ±0	246 ±53	0.21 ±0.04
PS1-C-FLAG	67 ±6	307 ±79	0.18 ±0.04
PS1-C-FLAG + 6myc-N-sel-10	67 ±6	302 ±94	0.20 ±0.08
APP695NL-KK	66 ±5	348 ±110	0.17 ±0.05
APP695NL-KK + 6myc-N-sel-10	75 ±18	448 ±141	0.15 ±0.03
APP695NL-KK + PS1-C-FLAG	63 ±26	466 ±72	0.12 ±0.02
APP695NL-KK + PS1-C-FLAG + 6myc-N-sel-10	81 ±26	565 ±179	0.12 ±0.01

Table 3. Endogenous and exogenous Ab1-40 and Ab1-42 levels in supernatants from stable transformants of HEK293 cells.

Stable Line	GFP Transfection		APP695NL-KK Transfection	
	Ab1-40 ng/ml	Ab1-42 ng/ml	Ab1-40 ng/ml	Ab1-42 ng/ml
6myc-N-sel10/2	297 ± 29	109 ± 17	4877 ± 547	750 ± 32
6myc-N-sel10/6	168 ± 18	85 ± 11	8310 ± 308	1391 ± 19
PS1-C-FLAG/2	97 ± 6	68 ± 8	3348 ± 68	493 ± 21
PS1-C-FLAG/8	118 ± 11	85 ± 17	3516 ± 364	515 ± 36
PS1-C-FLAG/9	83 ± 20	67 ± 16	2369 ± 73	350 ± 12
PS1-C-FLAG/11	152 ± 17	68 ± 13	4771 ± 325	599 ± 25
PS1-C-FLAG/12	141 ± 12	50 ± 10	4095 ± 210	449 ± 21
PS1-C-FLAG/13	270 ± 139	61 ± 28	6983 ± 304	745 ± 41
pcDNA3/1	43 ± 13	75 ± 15	1960 ± 234	61 ± 6

Table 4. Sel-10 constructs with epitope tags at the N or C terminus increase Aβ 1-40 and Aβ 1-42.

construct	Aβ 1-40	%increase	P-value	Aβ 1-42	%increase	P-value
pcDNA	4240 ± 102			614 ± 10		
6myc-N-sel-10	7631 ± 465	80%	3.7×10^{-6}	1136 ± 73	46%	7.9×10^{-6}
sel-10-C-mychis	5485 ± 329	29%	1.8×10^{-4}	795 ± 50	29%	4.0×10^{-4}
sel-10-C-V5his	6210 ± 498	46%	1.2×10^{-4}	906 ± 73	48%	2.1×10^{-4}

What is claimed is:

1. An isolated nucleic acid molecule comprising a polynucleotide having a sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a human sel-10 polypeptide having the complete amino acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7, or as encoded by the cDNA clone contained in ATCC Deposit No.98978;

(b) a nucleotide sequence encoding a human sel-10 polypeptide having the complete amino acid sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10, or as encoded by the cDNA clone contained in ATCC Deposit No. 98979; and

(c) a nucleotide sequence complementary to the nucleotide sequence of (a) or (b).

2. An isolated nucleic acid molecule comprising polynucleotide which hybridizes under stringent conditions to a polynucleotide having the nucleotide sequence in (a), (b), or (c) of claim 1.

3. The nucleic acid molecule of claim 1, wherein said polynucleotide of 1(a) encodes a human sel-10 polypeptide having the complete amino acid sequence of SEQ ID NO:3.

4. The nucleic acid molecule of claim 3, wherein said polynucleotide molecule of 1(a) comprises the nucleotide sequence of residues 45-1928 of SEQ ID NO:1.

5. The nucleic acid molecule of claim 1, wherein said polynucleotide of 1(a) encodes a human sel-10 polypeptide having the complete amino acid sequence of SEQ ID NO:4.

6. The nucleic acid molecule of claim 5, wherein said polynucleotide molecule of 1(a) comprises the nucleotide sequence of residues 150-1928 of SEQ ID NO:1.

7. The nucleic acid molecule of claim 1, wherein said polynucleotide of 1(a) encodes a human sel-10 polypeptide having the complete amino acid sequence of SEQ ID NO:5.

8. The nucleic acid molecule of claim 7, wherein said polynucleotide molecule of 1(a) comprises the nucleotide sequence of residues 267-1928 of SEQ ID NO:1.

9. The nucleic acid molecule of claim 1, wherein said polynucleotide of 1(a) encodes a human sel-10 polypeptide having the complete amino acid sequence of SEQ ID NO:6.

10 The nucleic acid molecule of claim 9, wherein said polynucleotide molecule of 1(a) comprises the nucleotide sequence of residues 291-1928 of SEQ ID NO:1.

11. The nucleic acid molecule of claim 1, wherein said polynucleotide of 1(a) encodes a human sel-10 polypeptide having the complete amino acid sequence of SEQ ID NO:7.

12. The nucleic acid molecule of claim 11, wherein said polynucleotide molecule of 1(a) comprises the nucleotide sequence of residues 306-1928 of SEQ ID NO:1.

13. The nucleic acid molecule of claim 1, wherein said polynucleotide of 1(b) encodes a human sel-10 polypeptide having the complete amino acid sequence of SEQ ID NO:8.

14. The nucleic acid molecule of claim 13 wherein said polynucleotide molecule of 1(b) comprises the nucleotide sequence of residues 180-1949 of SEQ ID NO:2.

15. The nucleic acid molecule of claim 1, wherein said polynucleotide of 1(b) encodes a human sel-10 polypeptide having the complete amino acid sequence of SEQ ID NO:9.

16. The nucleic acid molecule of claim 15, wherein said polynucleotide molecule of 1(b) comprises the nucleotide sequence of residues 270-1949 of SEQ ID NO:2.

17. The nucleic acid molecule of claim 1, wherein said polynucleotide of 1(b) encodes a human sel-10 polypeptide having the complete amino acid sequence of SEQ ID NO:10.

18. The nucleic acid molecule of claim 17, wherein said polynucleotide molecule of 1(b) comprises the nucleotide sequence of residues 327-1949 of SEQ ID NO:2.

19. A vector comprising the nucleic acid molecule of claim 1.

20. The vector of claim 19, wherein said nucleic acid molecule of claim 1 is operably linked to a promoter for the expression of a sel-10 polypeptide.

21. A host cell comprising the vector of claim 19.

22. The host cell of claim 21, wherein said host is a eukaryotic host.

23. A method of obtaining a sel-10 polypeptide comprising culturing the host cell of claim 22 and isolating said sel-10 polypeptide.

24. An isolated sel-10 polypeptide comprising

(a) an amino acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7, or as encoded by the cDNA clone contained in ATCC Deposit No. 98978;

(b) an amino acid sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10, or as encoded by the cDNA clone contained in ATCC Deposit No. 98979.

25. The isolated sel-10 polypeptide of claim 24, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO:3.

26. The isolated sel-10 polypeptide of claim 24, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO:4.

27. The isolated sel-10 polypeptide of claim 24, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO:5.

28. The isolated sel-10 polypeptide of claim 24, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO:6.

29. The isolated sel-10 polypeptide of claim 24, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO:7.

30. The isolated sel-10 polypeptide of claim 24, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO:8.

31. The isolated sel-10 polypeptide of claim 24, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO:9.

32. The isolated sel-10 polypeptide of claim 24, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO:10.

33. An isolated antibody that binds specifically to the sel-10 polypeptide of claim 24.

34. A cell line having altered $A\beta$ processing that expresses any of the sel-10 isolated nucleic acid molecules of claim 1.

35. The cell line of claim 34, wherein said $A\beta$ processing is increased.

36. The cell line of claim 34, wherein said $A\beta$ processing is decreased.

37. The cell line of claim 34, wherein said cell line is 6myc-N-sel10/2.

38. The cell line of claim 34, wherein said cell line is 6myc-N-sel10/6.

39. A method for the identification of an agent capable of altering the ratio of $A\beta_{1-40}/ A\beta_{1-40} + A\beta_{1-42}$ produced in any of the cell lines of claims 34, 37, and 38, comprising the steps of:

(a) obtaining a test culture and a control culture of said cell line;

(b) contacting said test culture with a test agent;

(c) measuring the levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ produced by said test culture of step (b) and said control culture;

(d) calculating the ratio of $A\beta_{1-40}/ A\beta_{1-40} + A\beta_{1-42}$ for said test culture and said control culture from the levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ measured in step (c); and

(e) comparing the ratio of $A\beta_{1-40}/ A\beta_{1-40} + A\beta_{1-42}$ measured for said test culture and said control culture in step (d);

whereby a determination that the ratio of $A\beta_{1-40}/ A\beta_{1-40} + A\beta_{1-42}$ for said test culture is higher or lower than ratio of $A\beta_{1-40}/ A\beta_{1-40} + A\beta_{1-42}$ for said control culture indicates that said test agent has altered the ratio of $A\beta_{1-40}/ A\beta_{1-40} + A\beta_{1-42}$.

40. The method of claim 39, wherein said ratio of $A\beta_{1-40}/ A\beta_{1-40} + A\beta_{1-42}$ is increased by said test agent.

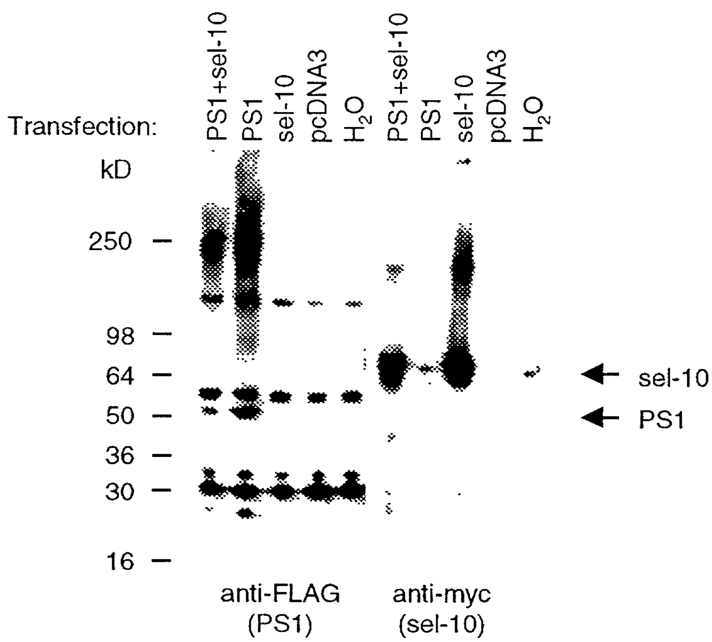
41. The method of claim 39, wherein said ratio of $A\beta_{1-40}/ A\beta_{1-40} + A\beta_{1-42}$ is decreased by said test agent.

Human Sel-10 Polypeptides and Polynucleotides that Encode Them

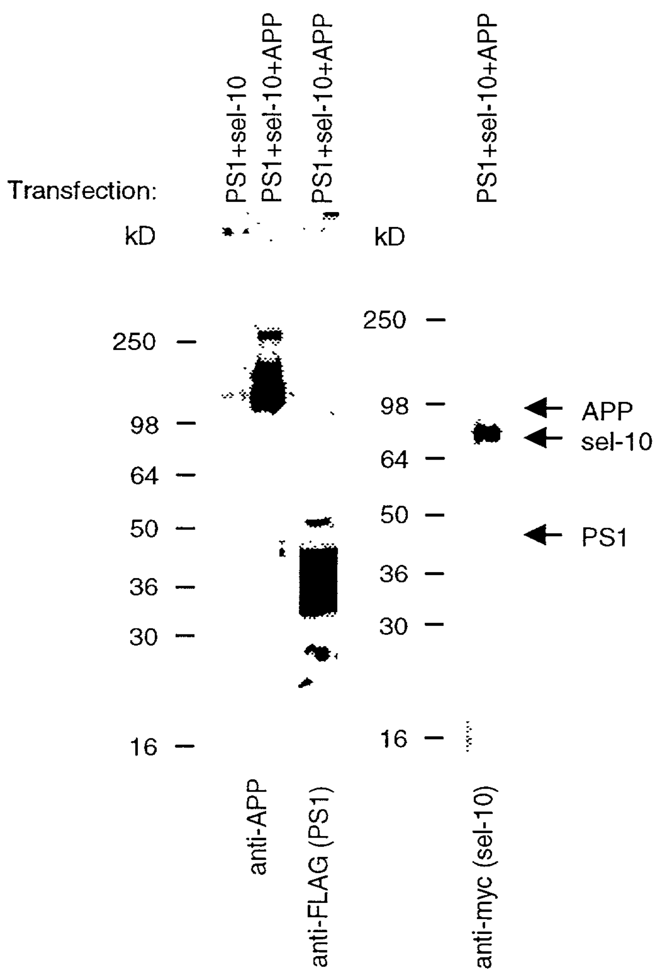
The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding either of two alternative splice variants of human sel-10, one of which is expressed in hippocampal cells, and one of which is expressed in mammary cells. The invention also provides isolated sel-10 polypeptides and cell lines which express them in which Aβ processing is altered.

FIGURES 1A AND 1B. Western blot of protein expression in HEK293 cells transfected with PS1-C-FLAG, 6-myc-N-sel-10, and APP295NL-KK cDNAs.

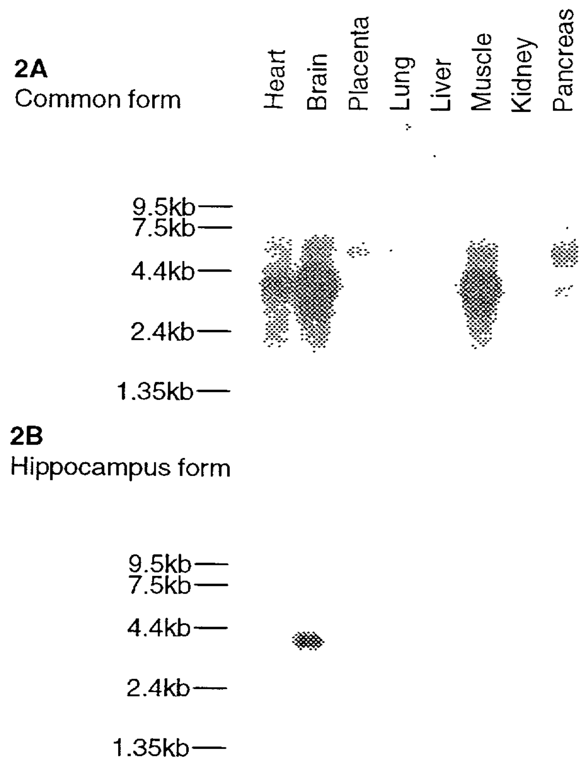
1A



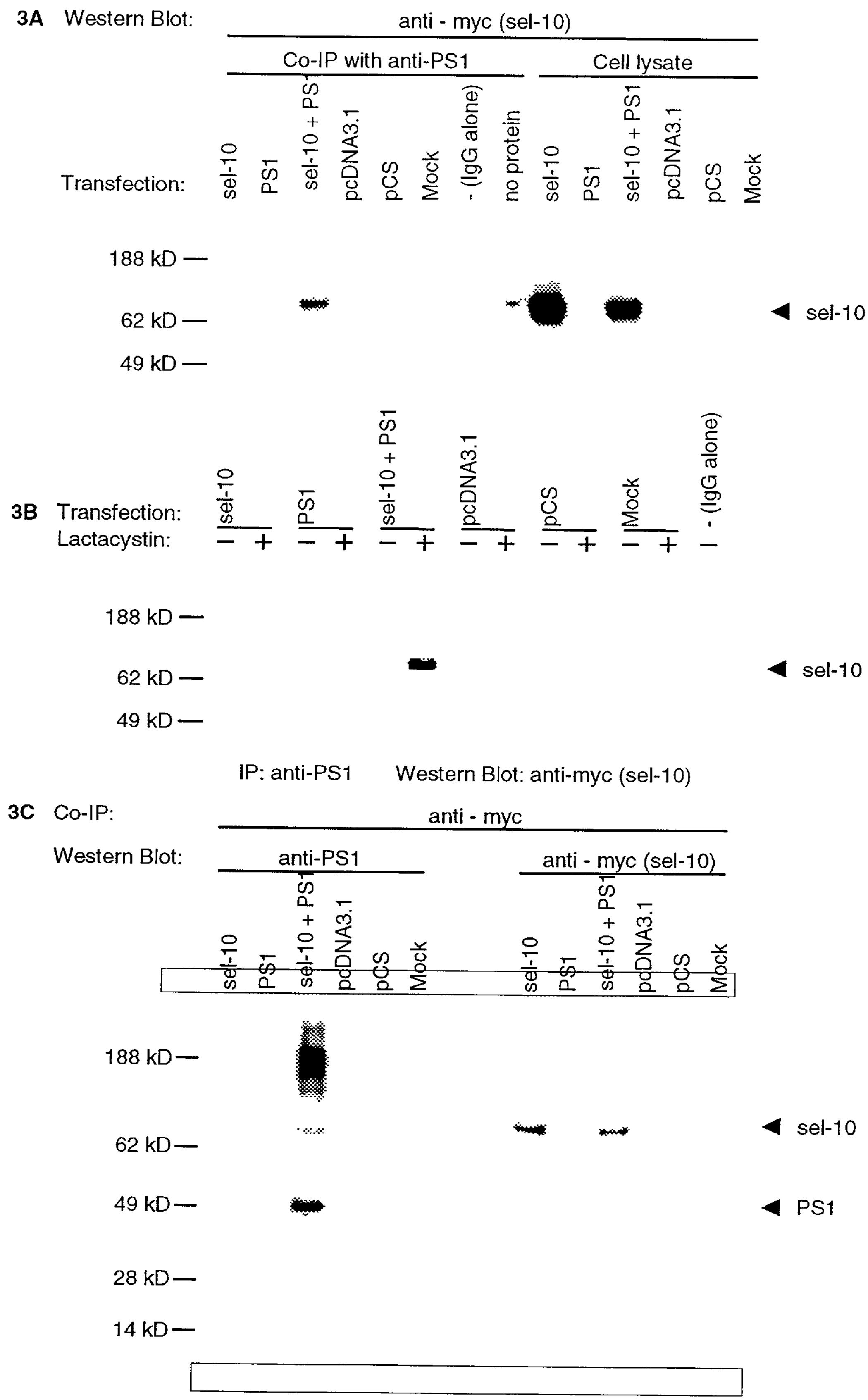
1B



FIGURES 2A & 2B

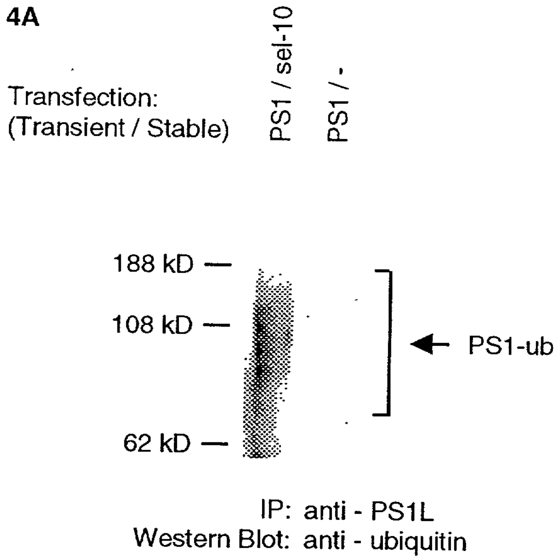


FIGURES 3A – 3C

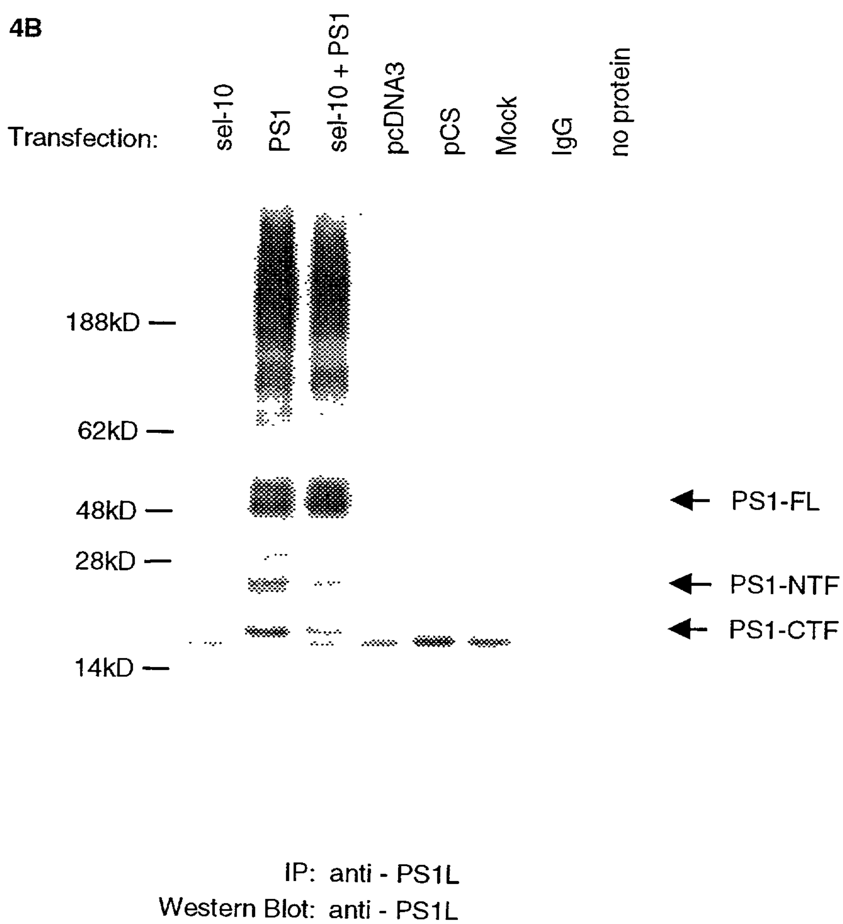


FIGURES 4A AND 4B

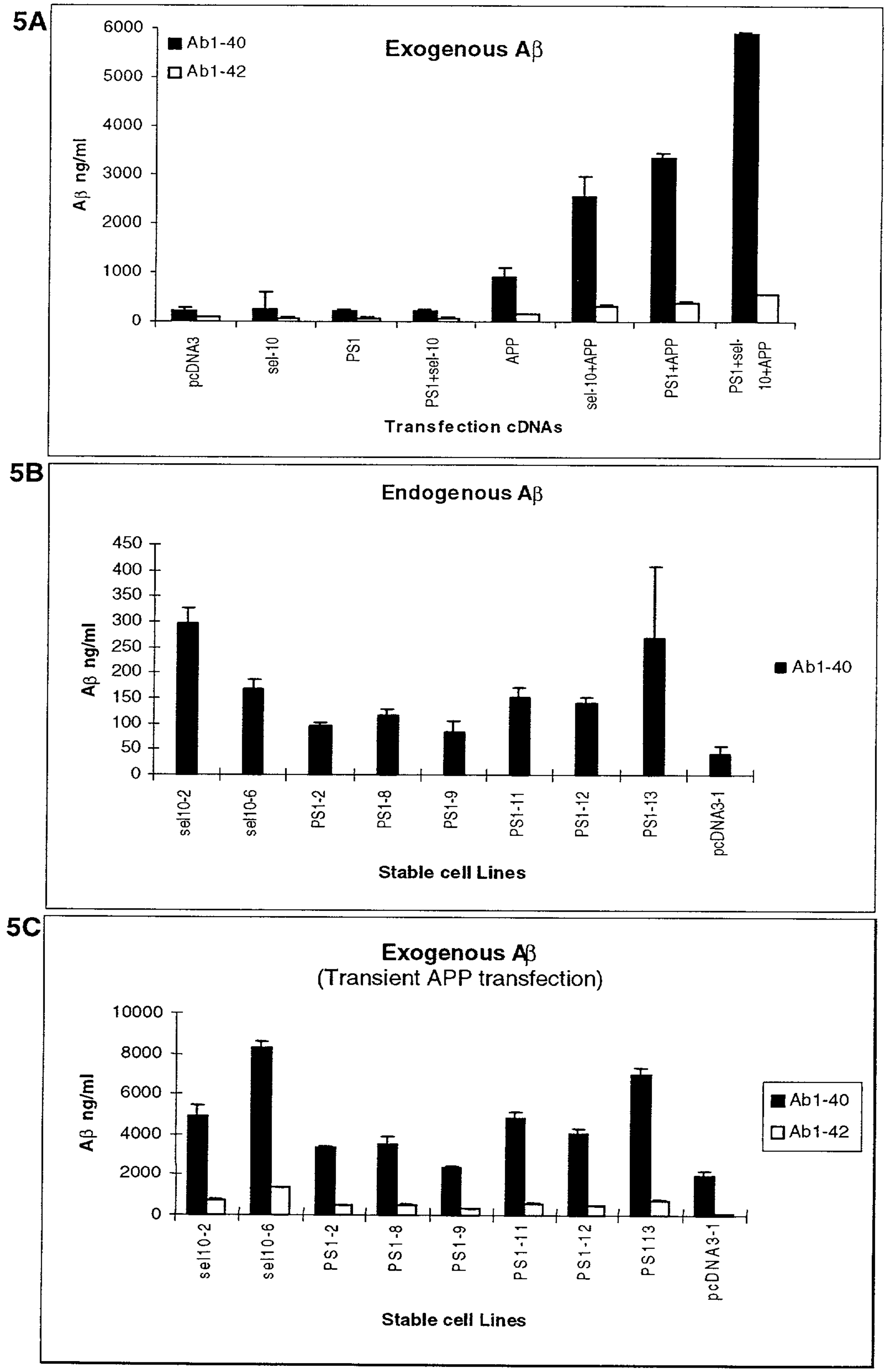
4A



4B



FIGURES 5A – 5C



SEQUENCE LISTING

<110> Gurney, Mark E.

Li, Jinhe

Pauley, Adele M.

Pharmacia & Upjohn Company

<120> Human Sel-10 Polypeptides and Polynucleotides that Encode Them

<130> 6142

<140> 6142

<141> 1997-12-19

<160> 27

<170> PatentIn Ver. 2.0

<210> 1

<211> 3550

<212> DNA

<213> Homo sapiens

<400> 1

ctcattattc cctcgagttc ttctcagtca agctgcatgt atgtatgtgt gtcccgagaa 60
gcggtttgat actgagctgc atttgccttt actgtggagt tttgttgccg gttctgctcc 120
ctaatcttcc ttttctgacg tgcctgagca tgtccacatt agaatctgtg acatacctac 180
ctgaaaaagg tttatattgt cagagactgc caagcagccg gacacacggg ggcacagaat 240
cactgaaggg gaaaaataca gaaaatatgg gtttctacgg cacattaaaa atgatttttt 300
acaaaatgaa aagaaagttg gaccatggtt ctgaggtccg ctctttttct ttgggaaaga 360
aaccatgcaa agtctcagaa tatacaagta ccactgggct tgtaccatgt tcagcaacac 420
caacaacttt tggggacctc agagcagcca atggccaagg gcaacaacga cgccgaatta 480
catctgtcca gccacctaca ggcctccagg aatggctaaa aatgtttcag agctggagtg 540
gaccagagaa attgcttgct ttagatgaac tcattgatag ttgtgaacca acacaagtaa 600
aacatatgat gcaagtgata gaaccccagt ttcaacgaga cttcatttca ttgctcccta 660

aagagttggc actctatgtg ctttcattcc tggaacccaa agacctgcta caagcagctc 720
agacatgtcg ctactggaga attttggctg aagacaacct tctctggaga gagaaatgca 780
aagaagaggg gattgatgaa ccattgcaca tcaagagaag aaaagtaata aaaccaggtt 840
tcatacacag tccatggaaa agtgcataca tcagacagca cagaattgat actaactgga 900
ggcgaggaga actcaaatct cctaaggtgc tgaaaggaca tgatgatcat gtgatcacat 960
gcttacagtt ttgtggtaac cgaatagtta gtggttctga tgacaacact ttaaaagttt 1020
ggtcagcagt cacaggcaaa tgtctgagaa cattagtggg acatacaggt ggagtatggt 1080
catcacaaat gagagacaac atcatcatta gtggatctac agatcggaca ctcaaagtgt 1140
ggaatgcaga gactggagaa tgtatacaca ccttatatgg gcatacttcc actgtgcgtt 1200
gtatgcatct tcatgaaaaa agagttgtta gcggttctcg agatgccact cttagggttt 1260
gggatattga gacaggccag tgtttacatg ttttgatggg tcatgttgca gcagtccgct 1320
gtgttcaata tgatggcagg agggttgtta gtggagcata tgattttatg gtaaaggtgt 1380
gggatccaga gactgaaacc tgtctacaca cgttgcaggg gcatactaat agagtctatt 1440
cattacagtt tgatggtatc catgtggtga gtggatctct tgatacatca atccgtgttt 1500
gggatgtgga gacagggaat tgcattcaca cgttaacagg gcaccagtcg ttaacaagtg 1560
gaatggaact caaagacaat attcttgtct ctgggaatgc agattctaca gttaaaatct 1620
gggatatcaa aacaggacag tgtttacaaa cattgcaagg tcccaacaag catcagagtg 1680
ctgtgacctg tttacagttc aacaagaact ttgtaattac cagctcagat gatggaactg 1740
taaaactatg ggacttgaaa acgggtgaat ttattcgaaa cctagtcaca ttggagagtg 1800
gggggagtgg gggagttgtg tggcggatca gagcctcaaa cacaaagctg gtgtgtgcag 1860
ttgggagtcg gaatgggact gaagaaacca agctgctggt gctggacttt gatgtggaca 1920
tgaagtgaag agcagaaaag atgaatttgt ccaattgtgt agacgatata ctccctgccc 1980
ttccccctgc aaaaagaaaa aaagaaaaga aaaagaaaaa aatcccttgt tctcagtggt 2040
gcaggatgtt ggcttggggc aacagattga aaagacctac agactaagaa ggaaaagaag 2100
aagagatgac aaaccataac tgacaagaga ggcgtctgct gtctcatcac ataaaaggct 2160
tcacttttga ctgagggcag ctttgcaaaa tgagactttc taaatcaaac caggtgcaat 2220
tatttcttta ttttcttctc cagtggtcat tggggcagtg ttaatgctga aacatcatta 2280
cagattctgc tagcctgttc ttttaccact gacagctaga cacctagaaa ggaactgcaa 2340
taatatcaaa acaagtactg gttgactttc taattagaga gcatctgcaa caaaaagtca 2400
tttttctgga gtggaaaagc ttaaaaaaat tactgtgaat tgtttttgta cagttatcat 2460
gaaaagcttt ttttttattt ttttngccaa ccattgccaa tgtcaatcaa tcacagtatt 2520
agcctctgtt aatctattta ctgttgcttc catatacatt cttcaatgca tatgttgctc 2580
aaaggtggca agttgtcctg ggttctgtga gtcctgagat ggatttaatt cttgatgctg 2640
gtgctagaag taggtcttca aatatgggat tgttgtccca accctgtact gtactcccag 2700

tggccaaact tatttatgct gctaaatgaa agaaagaaaa aagcaaatta ttttttttat 2760
ttttttctg ctgtgacgtt ttagtcccag actgaattcc aaatttgctc tagtttggtt 2820
atggaaaaaa gactttttgc cactgaaact tgagccatct gtgcctctaa gaggctgaga 2880
atggaagagt ttcagataat aaagagtgaa gtttgcctgc aagtaaagaa ttgagagtgt 2940
gtgcaaagct tattttcttt tatctgggca aaaattaaaa cacattcctt ggaacagagc 3000
tattacttgc ctgttctgtg gagaaacttt tctttttgag ggctgtggtg aatggatgaa 3060
cgtacatcgt aaaactgaca aaatatttta aaaatatata aaacacaaaa ttaaaataaa 3120
gttgctggtc agtcttagtg ttttacagta tttgggaaaa caactgttac agttttattg 3180
ctctgagtaa ctgacaaagc agaaactatt cagtttttgt agtaaaggcg tcacatgcaa 3240
acaaacaaaa tgaatgaaac agtcaaatgg tttgcctcat tctccaagag ccacaactca 3300
agctgaactg tgaaagtggt ttaacactgt atcctaggcg atctttttc ctccttctgt 3360
ttattttttt gnttgtttta tttatagtct gatttaaaac aatcagattc aagttggtta 3420
attttagtta tgtaacaacc tgacatgatg gaggaaaaca acctttaaag ggattgtgtc 3480
tatggtttga ttcacttaga aattttattt tcttataact taagtgcaat aaaatgtgtt 3540
ttttcatgtt 3550

<210> 2

<211> 3571

<212> DNA

<213> Homo sapiens

<400> 2

ctcagcaggt caggacattt ggtaggggaa ggttgaaaga caaaagcagc aggccttggg 60
ttctcagcct tttaaaaact attattaaat atatattttt aaaatttagt ggttagagct 120
tttagtaatg tgcctgtatt acatgtagag agtattcgtc aaccaagagg agttttaaaa 180
tgtcaaaacc gggaaaacct actctaaacc atggcttggt tcctgttgat cttaaaagtg 240
caaaagagcc tctaccacat caaaccgtga tgaagatatt tagcattagc atcattgccc 300
aaggcctccc tttttgtcga agacggatga aaagaaagtt ggaccatggt tctgaggtcc 360
gctcttttc tttgggaaag aaaccatgca aagtctcaga atatacaagt accactgggc 420
ttgtaccatg ttcagcaaca ccaacaactt ttggggacct cagagcagcc aatggccaag 480
ggcaacaacg acgccgaatt acatctgtcc agccacctac aggcctccag gaatggctaa 540
aaatgtttca gagctggagt ggaccagaga aattgcttgc tttagatgaa ctcattgata 600
gttgtgaacc aacacaagta aaacatatga tgcaagtgat agaaccccag tttcaacgag 660
acttcatttc attgctccct aaagagttgg cactctatgt gctttcattc ctggaaccca 720
aagacctgct acaagcagct cagacatgtc gctactggag aattttggct gaagacaacc 780

ttctctggag agagaaatgc aaagaagagg ggattgatga accattgcac atcaagagaa 840
gaaaagtaat aaaaccaggt ttcatacaca gtccatggaa aagtgcatac atcagacagc 900
acagaattga tactaactgg aggcgaggag aactcaaatc tcctaaggtg ctgaaaggac 960
atgatgatca tgtgatcaca tgcttacagt tttgtggtaa ccgaatagtt agtggttctg 1020
atgacaacac tttaaaagtt tggtcagcag tcacaggcaa atgtctgaga acattagtgg 1080
gacatacagg tggagtatgg tcatcacaaa tgagagacaa catcatcatt agtggatcta 1140
cagatcggac actcaaagtg tggaatgcag agactggaga atgtatacac accttatatg 1200
ggcatacttc cactgtgcgt tgtatgcatc ttcatgaaaa aagagttgtt agcggttctc 1260
gagatgccac tcttagggtt tgggatattg agacaggcca gtgtttacat gttttgatgg 1320
gtcatgttgc agcagtccgc tgtgttcaat atgatggcag gagggttgtt agtggagcat 1380
atgattttat ggtaaaggtg tgggatccag agactgaaac ctgtctacac acgttgcagg 1440
ggcatactaa tagagtctat tcattacagt ttgatggtat ccatgtggtg agtggatctc 1500
ttgatacatc aatccgtgtt tgggatgtgg agacagggaa ttgcattcac acgttaacag 1560
ggcaccagtc gttaacaagt ggaatggaac tcaaagacaa tattcttgtc tctgggaatg 1620
cagattctac agttaaaatc tgggatatca aaacaggaca gtgtttacaa acattgcaag 1680
gtcccaacaa gcatcagagt gctgtgacct gtttacagtt caacaagaac tttgtaatta 1740
ccagctcaga tgatggaact gtaaaactat gggacttgaa aacgggtgaa tttattcgaa 1800
acctagtcac attggagagt gggggagtgg ggggagttgt gtggcggatc agagcctcaa 1860
acacaaagct ggtgtgtgca gttgggagtc ggaatgggac tgaagaaacc aagctgctgg 1920
tgctggactt tgatgtggac atgaagtgaa gagcagaaaa gatgaatttg tccaattgtg 1980
tagacgatat actccctgcc cttccccctg caaaaagaaa aaaagaaaag aaaaagaaaa 2040
aaatcccttg ttctcagtgg tgcaggatgt tggcttgggg caacagattg aaaagaccta 2100
cagactaaga aggaaaagaa gaagagatga caaaccataa ctgacaagag aggcgtctgc 2160
tgtctcatca cataaaaggc ttcacttttg actgagggca gctttgcaaa atgagacttt 2220
ctaaatcaaa ccaggtgcaa ttatttcttt attttcttct ccagtggtca ttggggcagt 2280
gttaatgctg aaacatcatt acagattctg ctagcctgtt cttttaccac tgacagctag 2340
acacctagaa aggaactgca ataatatcaa aacaagtact ggttgacttt ctaattagag 2400
agcatctgca acaaaaagtc atttttctgg agtggaaaag cttaaaaaaa ttactgtgaa 2460
ttgtttttgt acagttatca tgaaaagctt ttttttttat tttttngcca accattgcca 2520
atgtcaatca atcacagtat tagcctctgt taatctattt actgttgctt ccatatacat 2580
tcttcaatgc atatgttgct caaaggtggc aagttgtcct gggttctgtg agtcctgaga 2640
tggatttaat tcttgatgct ggtgctagaa gtaggtcttc aaatatggga ttgttgtccc 2700
aaccctgtac tgtactccca gtggccaaac ttatttatgc tgctaaatga aagaaagaaa 2760
aaagcaaatt attttttta tttttttct gctgtgacgt tttagtccca gactgaattc 2820

caaatttgct ctagtttggt tatggaaaaa agactttttg ccactgaaac ttgagccatc 2880

tgtgcctcta agaggctgag aatggaagag tttcagataa taaagagtga agtttgcctg 2940

caagtaaaga attgagagtg tgtgcaaagc ttattttctt ttatctgggc aaaaattaaa 3000

acacattcct tggaacagag ctattacttg cctgttctgt ggagaaactt ttctttttga 3060

gggctgtggt gaatggatga acgtacatcg taaaactgac aaaatatttt aaaaatatat 3120

aaaacacaaa attaaaataa agttgctggt cagtcttagt gttttacagt atttgggaaa 3180

acaactgtta cagttttatt gctctgagta actgacaaag cagaaactat tcagtttttg 3240

tagtaaaggc gtcacatgca aacaaacaaa atgaatgaaa cagtcaaatg gtttgcctca 3300

ttctccaaga gccacaactc aagctgaact gtgaaagtgg tttaacactg tatcctaggc 3360

gatcttttt cctccttctg tttattttt tgnttgtttt atttatagtc tgatttaaaa 3420

caatcagatt caagttggtt aattttagtt atgtaacaac ctgacatgat ggaggaaaac 3480

aacctttaaa gggattgtgt ctatggtttg attcacttag aaattttatt ttcttataac 3540

ttaagtgcaa taaaatgtgt tttttcatgt t 3571

<210> 3

<211> 627

<212> PRT

<213> Homo sapiens

<400> 3

Met Cys Val Pro Arg Ser Gly Leu Ile Leu Ser Cys Ile Cys Leu Tyr
1 5 10 15

Cys Gly Val Leu Leu Pro Val Leu Leu Pro Asn Leu Pro Phe Leu Thr
20 25 30

Cys Leu Ser Met Ser Thr Leu Glu Ser Val Thr Tyr Leu Pro Glu Lys
35 40 45

Gly Leu Tyr Cys Gln Arg Leu Pro Ser Ser Arg Thr His Gly Gly Thr
50 55 60

Glu Ser Leu Lys Gly Lys Asn Thr Glu Asn Met Gly Phe Tyr Gly Thr
65 70 75 80

Leu Lys Met Ile Phe Tyr Lys Met Lys Arg Lys Leu Asp His Gly Ser

85 90 95

Glu Val Arg Ser Phe Ser Leu Gly Lys Lys Pro Cys Lys Val Ser Glu

100 105 110

Tyr Thr Ser Thr Thr Gly Leu Val Pro Cys Ser Ala Thr Pro Thr Thr

115 120 125

Phe Gly Asp Leu Arg Ala Ala Asn Gly Gln Gly Gln Gln Arg Arg Arg

130 135 140

Ile Thr Ser Val Gln Pro Pro Thr Gly Leu Gln Glu Trp Leu Lys Met

145 150 155 160

Phe Gln Ser Trp Ser Gly Pro Glu Lys Leu Leu Ala Leu Asp Glu Leu

165 170 175

Ile Asp Ser Cys Glu Pro Thr Gln Val Lys His Met Met Gln Val Ile

180 185 190

Glu Pro Gln Phe Gln Arg Asp Phe Ile Ser Leu Leu Pro Lys Glu Leu

195 200 205

Ala Leu Tyr Val Leu Ser Phe Leu Glu Pro Lys Asp Leu Leu Gln Ala

210 215 220

Ala Gln Thr Cys Arg Tyr Trp Arg Ile Leu Ala Glu Asp Asn Leu Leu

225 230 235 240

Trp Arg Glu Lys Cys Lys Glu Glu Gly Ile Asp Glu Pro Leu His Ile

245 250 255

Lys Arg Arg Lys Val Ile Lys Pro Gly Phe Ile His Ser Pro Trp Lys

260 265 270

Ser Ala Tyr Ile Arg Gln His Arg Ile Asp Thr Asn Trp Arg Arg Gly

275 280 285

Glu Leu Lys Ser Pro Lys Val Leu Lys Gly His Asp Asp His Val Ile

290 295 300

Thr Cys Leu Gln Phe Cys Gly Asn Arg Ile Val Ser Gly Ser Asp Asp

305 310 315 320

Asn Thr Leu Lys Val Trp Ser Ala Val Thr Gly Lys Cys Leu Arg Thr

325 330 335

Leu Val Gly His Thr Gly Gly Val Trp Ser Ser Gln Met Arg Asp Asn

340 345 350

Ile Ile Ile Ser Gly Ser Thr Asp Arg Thr Leu Lys Val Trp Asn Ala

355 360 365

Glu Thr Gly Glu Cys Ile His Thr Leu Tyr Gly His Thr Ser Thr Val

370 375 380

Arg Cys Met His Leu His Glu Lys Arg Val Val Ser Gly Ser Arg Asp

385 390 395 400

Ala Thr Leu Arg Val Trp Asp Ile Glu Thr Gly Gln Cys Leu His Val

405 410 415

Leu Met Gly His Val Ala Ala Val Arg Cys Val Gln Tyr Asp Gly Arg

420 425 430

Arg Val Val Ser Gly Ala Tyr Asp Phe Met Val Lys Val Trp Asp Pro

435 440 445

Glu Thr Glu Thr Cys Leu His Thr Leu Gln Gly His Thr Asn Arg Val

450 455 460

Tyr Ser Leu Gln Phe Asp Gly Ile His Val Val Ser Gly Ser Leu Asp

465 470 475 480

Thr Ser Ile Arg Val Trp Asp Val Glu Thr Gly Asn Cys Ile His Thr

485 490 495

Leu Thr Gly His Gln Ser Leu Thr Ser Gly Met Glu Leu Lys Asp Asn

500 505 510

Ile Leu Val Ser Gly Asn Ala Asp Ser Thr Val Lys Ile Trp Asp Ile

515 520 525

Lys Thr Gly Gln Cys Leu Gln Thr Leu Gln Gly Pro Asn Lys His Gln

530 535 540

Ser Ala Val Thr Cys Leu Gln Phe Asn Lys Asn Phe Val Ile Thr Ser

545 550 555 560

Ser Asp Asp Gly Thr Val Lys Leu Trp Asp Leu Lys Thr Gly Glu Phe

565 570 575

Ile Arg Asn Leu Val Thr Leu Glu Ser Gly Gly Ser Gly Gly Val Val

580 585 590

Trp Arg Ile Arg Ala Ser Asn Thr Lys Leu Val Cys Ala Val Gly Ser

595 600 605

Arg Asn Gly Thr Glu Glu Thr Lys Leu Leu Val Leu Asp Phe Asp Val

610 615 620

Asp Met Lys

625

<210> 4

<211> 592

<212> PRT

<213> Homo sapiens

<400> 4

Met Ser Thr Leu Glu Ser Val Thr Tyr Leu Pro Glu Lys Gly Leu Tyr
1 5 10 15

Cys Gln Arg Leu Pro Ser Ser Arg Thr His Gly Gly Thr Glu Ser Leu
20 25 30

Lys Gly Lys Asn Thr Glu Asn Met Gly Phe Tyr Gly Thr Leu Lys Met
35 40 45

Ile Phe Tyr Lys Met Lys Arg Lys Leu Asp His Gly Ser Glu Val Arg
50 55 60

Ser Phe Ser Leu Gly Lys Lys Pro Cys Lys Val Ser Glu Tyr Thr Ser
65 70 75 80

Thr Thr Gly Leu Val Pro Cys Ser Ala Thr Pro Thr Thr Phe Gly Asp
85 90 95

Leu Arg Ala Ala Asn Gly Gln Gly Gln Gln Arg Arg Arg Ile Thr Ser
100 105 110

Val Gln Pro Pro Thr Gly Leu Gln Glu Trp Leu Lys Met Phe Gln Ser
115 120 125

Trp Ser Gly Pro Glu Lys Leu Leu Ala Leu Asp Glu Leu Ile Asp Ser
130 135 140

Cys Glu Pro Thr Gln Val Lys His Met Met Gln Val Ile Glu Pro Gln

145   150   155   160

Phe Gln Arg Asp Phe Ile Ser Leu Leu Pro Lys Glu Leu Ala Leu Tyr

                165   170   175

Val Leu Ser Phe Leu Glu Pro Lys Asp Leu Leu Gln Ala Ala Gln Thr

            180   185   190

Cys Arg Tyr Trp Arg Ile Leu Ala Glu Asp Asn Leu Leu Trp Arg Glu

        195   200   205

Lys Cys Lys Glu Glu Gly Ile Asp Glu Pro Leu His Ile Lys Arg Arg

    210   215   220

Lys Val Ile Lys Pro Gly Phe Ile His Ser Pro Trp Lys Ser Ala Tyr

225   230   235   240

Ile Arg Gln His Arg Ile Asp Thr Asn Trp Arg Arg Gly Glu Leu Lys

                245   250   255

Ser Pro Lys Val Leu Lys Gly His Asp Asp His Val Ile Thr Cys Leu

            260   265   270

Gln Phe Cys Gly Asn Arg Ile Val Ser Gly Ser Asp Asp Asn Thr Leu

        275   280   285

Lys Val Trp Ser Ala Val Thr Gly Lys Cys Leu Arg Thr Leu Val Gly

    290   295   300

His Thr Gly Gly Val Trp Ser Ser Gln Met Arg Asp Asn Ile Ile Ile

305   310   315   320

Ser Gly Ser Thr Asp Arg Thr Leu Lys Val Trp Asn Ala Glu Thr Gly
325 330 335

Glu Cys Ile His Thr Leu Tyr Gly His Thr Ser Thr Val Arg Cys Met
340 345 350

His Leu His Glu Lys Arg Val Val Ser Gly Ser Arg Asp Ala Thr Leu
355 360 365

Arg Val Trp Asp Ile Glu Thr Gly Gln Cys Leu His Val Leu Met Gly
370 375 380

His Val Ala Ala Val Arg Cys Val Gln Tyr Asp Gly Arg Arg Val Val
385 390 395 400

Ser Gly Ala Tyr Asp Phe Met Val Lys Val Trp Asp Pro Glu Thr Glu
405 410 415

Thr Cys Leu His Thr Leu Gln Gly His Thr Asn Arg Val Tyr Ser Leu
420 425 430

Gln Phe Asp Gly Ile His Val Val Ser Gly Ser Leu Asp Thr Ser Ile
435 440 445

Arg Val Trp Asp Val Glu Thr Gly Asn Cys Ile His Thr Leu Thr Gly
450 455 460

His Gln Ser Leu Thr Ser Gly Met Glu Leu Lys Asp Asn Ile Leu Val
465 470 475 480

Ser Gly Asn Ala Asp Ser Thr Val Lys Ile Trp Asp Ile Lys Thr Gly
485 490 495

Gln Cys Leu Gln Thr Leu Gln Gly Pro Asn Lys His Gln Ser Ala Val
500 505 510

Thr Cys Leu Gln Phe Asn Lys Asn Phe Val Ile Thr Ser Ser Asp Asp

515 520 525

Gly Thr Val Lys Leu Trp Asp Leu Lys Thr Gly Glu Phe Ile Arg Asn

530 535 540

Leu Val Thr Leu Glu Ser Gly Gly Ser Gly Gly Val Val Trp Arg Ile

545 550 555 560

Arg Ala Ser Asn Thr Lys Leu Val Cys Ala Val Gly Ser Arg Asn Gly

565 570 575

Thr Glu Glu Thr Lys Leu Leu Val Leu Asp Phe Asp Val Asp Met Lys

580 585 590

<210> 5

<211> 553

<212> PRT

<213> Homo sapiens

<400> 5

Met Gly Phe Tyr Gly Thr Leu Lys Met Ile Phe Tyr Lys Met Lys Arg

1 5 10 15

Lys Leu Asp His Gly Ser Glu Val Arg Ser Phe Ser Leu Gly Lys Lys

20 25 30

Pro Cys Lys Val Ser Glu Tyr Thr Ser Thr Thr Gly Leu Val Pro Cys

35 40 45

Ser Ala Thr Pro Thr Thr Phe Gly Asp Leu Arg Ala Ala Asn Gly Gln
50 55 60

Gly Gln Gln Arg Arg Arg Ile Thr Ser Val Gln Pro Pro Thr Gly Leu
65 70 75 80

Gln Glu Trp Leu Lys Met Phe Gln Ser Trp Ser Gly Pro Glu Lys Leu
85 90 95

Leu Ala Leu Asp Glu Leu Ile Asp Ser Cys Glu Pro Thr Gln Val Lys
100 105 110

His Met Met Gln Val Ile Glu Pro Gln Phe Gln Arg Asp Phe Ile Ser
115 120 125

Leu Leu Pro Lys Glu Leu Ala Leu Tyr Val Leu Ser Phe Leu Glu Pro
130 135 140

Lys Asp Leu Leu Gln Ala Ala Gln Thr Cys Arg Tyr Trp Arg Ile Leu
145 150 155 160

Ala Glu Asp Asn Leu Leu Trp Arg Glu Lys Cys Lys Glu Glu Gly Ile
165 170 175

Asp Glu Pro Leu His Ile Lys Arg Arg Lys Val Ile Lys Pro Gly Phe
180 185 190

Ile His Ser Pro Trp Lys Ser Ala Tyr Ile Arg Gln His Arg Ile Asp
195 200 205

Thr Asn Trp Arg Arg Gly Glu Leu Lys Ser Pro Lys Val Leu Lys Gly
210 215 220

His Asp Asp His Val Ile Thr Cys Leu Gln Phe Cys Gly Asn Arg Ile

225 230 235 240

Val Ser Gly Ser Asp Asp Asn Thr Leu Lys Val Trp Ser Ala Val Thr

245 250 255

Gly Lys Cys Leu Arg Thr Leu Val Gly His Thr Gly Gly Val Trp Ser

260 265 270

Ser Gln Met Arg Asp Asn Ile Ile Ile Ser Gly Ser Thr Asp Arg Thr

275 280 285

Leu Lys Val Trp Asn Ala Glu Thr Gly Glu Cys Ile His Thr Leu Tyr

290 295 300

Gly His Thr Ser Thr Val Arg Cys Met His Leu His Glu Lys Arg Val

305 310 315 320

Val Ser Gly Ser Arg Asp Ala Thr Leu Arg Val Trp Asp Ile Glu Thr

325 330 335

Gly Gln Cys Leu His Val Leu Met Gly His Val Ala Ala Val Arg Cys

340 345 350

Val Gln Tyr Asp Gly Arg Arg Val Val Ser Gly Ala Tyr Asp Phe Met

355 360 365

Val Lys Val Trp Asp Pro Glu Thr Glu Thr Cys Leu His Thr Leu Gln

370 375 380

Gly His Thr Asn Arg Val Tyr Ser Leu Gln Phe Asp Gly Ile His Val

385 390 395 400

Val Ser Gly Ser Leu Asp Thr Ser Ile Arg Val Trp Asp Val Glu Thr

405 410 415

Gly Asn Cys Ile His Thr Leu Thr Gly His Gln Ser Leu Thr Ser Gly

420 425 430

Met Glu Leu Lys Asp Asn Ile Leu Val Ser Gly Asn Ala Asp Ser Thr

435 440 445

Val Lys Ile Trp Asp Ile Lys Thr Gly Gln Cys Leu Gln Thr Leu Gln

450 455 460

Gly Pro Asn Lys His Gln Ser Ala Val Thr Cys Leu Gln Phe Asn Lys

465 470 475 480

Asn Phe Val Ile Thr Ser Ser Asp Asp Gly Thr Val Lys Leu Trp Asp

485 490 495

Leu Lys Thr Gly Glu Phe Ile Arg Asn Leu Val Thr Leu Glu Ser Gly

500 505 510

Gly Ser Gly Gly Val Val Trp Arg Ile Arg Ala Ser Asn Thr Lys Leu

515 520 525

Val Cys Ala Val Gly Ser Arg Asn Gly Thr Glu Glu Thr Lys Leu Leu

530 535 540

Val Leu Asp Phe Asp Val Asp Met Lys

545 550

<210> 6

<211> 545

<212> PRT

<213> Homo sapiens

<400> 6

Met Ile Phe Tyr Lys Met Lys Arg Lys Leu Asp His Gly Ser Glu Val
1 5 10 15

Arg Ser Phe Ser Leu Gly Lys Lys Pro Cys Lys Val Ser Glu Tyr Thr
20 25 30

Ser Thr Thr Gly Leu Val Pro Cys Ser Ala Thr Pro Thr Thr Phe Gly
35 40 45

Asp Leu Arg Ala Ala Asn Gly Gln Gly Gln Gln Arg Arg Arg Ile Thr
50 55 60

Ser Val Gln Pro Pro Thr Gly Leu Gln Glu Trp Leu Lys Met Phe Gln
65 70 75 80

Ser Trp Ser Gly Pro Glu Lys Leu Leu Ala Leu Asp Glu Leu Ile Asp
85 90 95

Ser Cys Glu Pro Thr Gln Val Lys His Met Met Gln Val Ile Glu Pro
100 105 110

Gln Phe Gln Arg Asp Phe Ile Ser Leu Leu Pro Lys Glu Leu Ala Leu
115 120 125

Tyr Val Leu Ser Phe Leu Glu Pro Lys Asp Leu Leu Gln Ala Ala Gln
130 135 140

Thr Cys Arg Tyr Trp Arg Ile Leu Ala Glu Asp Asn Leu Leu Trp Arg
145 150 155 160

Glu Lys Cys Lys Glu Glu Gly Ile Asp Glu Pro Leu His Ile Lys Arg
165 170 175

Arg Lys Val Ile Lys Pro Gly Phe Ile His Ser Pro Trp Lys Ser Ala

180 185 190

Tyr Ile Arg Gln His Arg Ile Asp Thr Asn Trp Arg Arg Gly Glu Leu

195 200 205

Lys Ser Pro Lys Val Leu Lys Gly His Asp Asp His Val Ile Thr Cys

210 215 220

Leu Gln Phe Cys Gly Asn Arg Ile Val Ser Gly Ser Asp Asp Asn Thr

225 230 235 240

Leu Lys Val Trp Ser Ala Val Thr Gly Lys Cys Leu Arg Thr Leu Val

245 250 255

Gly His Thr Gly Gly Val Trp Ser Ser Gln Met Arg Asp Asn Ile Ile

260 265 270

Ile Ser Gly Ser Thr Asp Arg Thr Leu Lys Val Trp Asn Ala Glu Thr

275 280 285

Gly Glu Cys Ile His Thr Leu Tyr Gly His Thr Ser Thr Val Arg Cys

290 295 300

Met His Leu His Glu Lys Arg Val Val Ser Gly Ser Arg Asp Ala Thr

305 310 315 320

Leu Arg Val Trp Asp Ile Glu Thr Gly Gln Cys Leu His Val Leu Met

325 330 335

Gly His Val Ala Ala Val Arg Cys Val Gln Tyr Asp Gly Arg Arg Val

340 345 350

Val Ser Gly Ala Tyr Asp Phe Met Val Lys Val Trp Asp Pro Glu Thr

355 360 365

Glu Thr Cys Leu His Thr Leu Gln Gly His Thr Asn Arg Val Tyr Ser

370 375 380

Leu Gln Phe Asp Gly Ile His Val Val Ser Gly Ser Leu Asp Thr Ser

385 390 395 400

Ile Arg Val Trp Asp Val Glu Thr Gly Asn Cys Ile His Thr Leu Thr

405 410 415

Gly His Gln Ser Leu Thr Ser Gly Met Glu Leu Lys Asp Asn Ile Leu

420 425 430

Val Ser Gly Asn Ala Asp Ser Thr Val Lys Ile Trp Asp Ile Lys Thr

435 440 445

Gly Gln Cys Leu Gln Thr Leu Gln Gly Pro Asn Lys His Gln Ser Ala

450 455 460

Val Thr Cys Leu Gln Phe Asn Lys Asn Phe Val Ile Thr Ser Ser Asp

465 470 475 480

Asp Gly Thr Val Lys Leu Trp Asp Leu Lys Thr Gly Glu Phe Ile Arg

485 490 495

Asn Leu Val Thr Leu Glu Ser Gly Gly Ser Gly Gly Val Val Trp Arg

500 505 510

Ile Arg Ala Ser Asn Thr Lys Leu Val Cys Ala Val Gly Ser Arg Asn

515 520 525

Gly Thr Glu Glu Thr Lys Leu Leu Val Leu Asp Phe Asp Val Asp Met

530 535 540

Lys

545

<210> 7

<211> 540

<212> PRT

<213> Homo sapiens

<400> 7

Met Lys Arg Lys Leu Asp His Gly Ser Glu Val Arg Ser Phe Ser Leu
1 5 10 15

Gly Lys Lys Pro Cys Lys Val Ser Glu Tyr Thr Ser Thr Thr Gly Leu
20 25 30

Val Pro Cys Ser Ala Thr Pro Thr Thr Phe Gly Asp Leu Arg Ala Ala
35 40 45

Asn Gly Gln Gly Gln Gln Arg Arg Arg Ile Thr Ser Val Gln Pro Pro
50 55 60

Thr Gly Leu Gln Glu Trp Leu Lys Met Phe Gln Ser Trp Ser Gly Pro
65 70 75 80

Glu Lys Leu Leu Ala Leu Asp Glu Leu Ile Asp Ser Cys Glu Pro Thr
85 90 95

Gln Val Lys His Met Met Gln Val Ile Glu Pro Gln Phe Gln Arg Asp
100 105 110

Phe Ile Ser Leu Leu Pro Lys Glu Leu Ala Leu Tyr Val Leu Ser Phe
115 120 125

Leu Glu Pro Lys Asp Leu Leu Gln Ala Ala Gln Thr Cys Arg Tyr Trp
130 135 140

Arg Ile Leu Ala Glu Asp Asn Leu Leu Trp Arg Glu Lys Cys Lys Glu

145 150 155 160

Glu Gly Ile Asp Glu Pro Leu His Ile Lys Arg Arg Lys Val Ile Lys

165 170 175

Pro Gly Phe Ile His Ser Pro Trp Lys Ser Ala Tyr Ile Arg Gln His

180 185 190

Arg Ile Asp Thr Asn Trp Arg Arg Gly Glu Leu Lys Ser Pro Lys Val

195 200 205

Leu Lys Gly His Asp Asp His Val Ile Thr Cys Leu Gln Phe Cys Gly

210 215 220

Asn Arg Ile Val Ser Gly Ser Asp Asp Asn Thr Leu Lys Val Trp Ser

225 230 235 240

Ala Val Thr Gly Lys Cys Leu Arg Thr Leu Val Gly His Thr Gly Gly

245 250 255

Val Trp Ser Ser Gln Met Arg Asp Asn Ile Ile Ile Ser Gly Ser Thr

260 265 270

Asp Arg Thr Leu Lys Val Trp Asn Ala Glu Thr Gly Glu Cys Ile His

275 280 285

Thr Leu Tyr Gly His Thr Ser Thr Val Arg Cys Met His Leu His Glu

290 295 300

Lys Arg Val Val Ser Gly Ser Arg Asp Ala Thr Leu Arg Val Trp Asp

305 310 315 320

Ile Glu Thr Gly Gln Cys Leu His Val Leu Met Gly His Val Ala Ala

325 330 335

Val Arg Cys Val Gln Tyr Asp Gly Arg Arg Val Val Ser Gly Ala Tyr

340 345 350

Asp Phe Met Val Lys Val Trp Asp Pro Glu Thr Glu Thr Cys Leu His

355 360 365

Thr Leu Gln Gly His Thr Asn Arg Val Tyr Ser Leu Gln Phe Asp Gly

370 375 380

Ile His Val Val Ser Gly Ser Leu Asp Thr Ser Ile Arg Val Trp Asp

385 390 395 400

Val Glu Thr Gly Asn Cys Ile His Thr Leu Thr Gly His Gln Ser Leu

405 410 415

Thr Ser Gly Met Glu Leu Lys Asp Asn Ile Leu Val Ser Gly Asn Ala

420 425 430

Asp Ser Thr Val Lys Ile Trp Asp Ile Lys Thr Gly Gln Cys Leu Gln

435 440 445

Thr Leu Gln Gly Pro Asn Lys His Gln Ser Ala Val Thr Cys Leu Gln

450 455 460

Phe Asn Lys Asn Phe Val Ile Thr Ser Ser Asp Asp Gly Thr Val Lys

465 470 475 480

Leu Trp Asp Leu Lys Thr Gly Glu Phe Ile Arg Asn Leu Val Thr Leu

485 490 495

Glu Ser Gly Gly Ser Gly Gly Val Val Trp Arg Ile Arg Ala Ser Asn

500 505 510

Thr Lys Leu Val Cys Ala Val Gly Ser Arg Asn Gly Thr Glu Glu Thr

515 520 525

Lys Leu Leu Val Leu Asp Phe Asp Val Asp Met Lys

530 535 540

<210> 8

<211> 589

<212> PRT

<213> Homo sapiens

<400> 8

Met Ser Lys Pro Gly Lys Pro Thr Leu Asn His Gly Leu Val Pro Val

1 5 10 15

Asp Leu Lys Ser Ala Lys Glu Pro Leu Pro His Gln Thr Val Met Lys

20 25 30

Ile Phe Ser Ile Ser Ile Ile Ala Gln Gly Leu Pro Phe Cys Arg Arg

35 40 45

Arg Met Lys Arg Lys Leu Asp His Gly Ser Glu Val Arg Ser Phe Ser

50 55 60

Leu Gly Lys Lys Pro Cys Lys Val Ser Glu Tyr Thr Ser Thr Thr Gly

65 70 75 80

Leu Val Pro Cys Ser Ala Thr Pro Thr Thr Phe Gly Asp Leu Arg Ala

85 90 95

Ala Asn Gly Gln Gly Gln Gln Arg Arg Arg Ile Thr Ser Val Gln Pro

100 105 110

Pro Thr Gly Leu Gln Glu Trp Leu Lys Met Phe Gln Ser Trp Ser Gly

115 120 125

Pro Glu Lys Leu Leu Ala Leu Asp Glu Leu Ile Asp Ser Cys Glu Pro

130 135 140

Thr Gln Val Lys His Met Met Gln Val Ile Glu Pro Gln Phe Gln Arg

145 150 155 160

Asp Phe Ile Ser Leu Leu Pro Lys Glu Leu Ala Leu Tyr Val Leu Ser

165 170 175

Phe Leu Glu Pro Lys Asp Leu Leu Gln Ala Ala Gln Thr Cys Arg Tyr

180 185 190

Trp Arg Ile Leu Ala Glu Asp Asn Leu Leu Trp Arg Glu Lys Cys Lys

195 200 205

Glu Glu Gly Ile Asp Glu Pro Leu His Ile Lys Arg Arg Lys Val Ile

210 215 220

Lys Pro Gly Phe Ile His Ser Pro Trp Lys Ser Ala Tyr Ile Arg Gln

225 230 235 240

His Arg Ile Asp Thr Asn Trp Arg Arg Gly Glu Leu Lys Ser Pro Lys

245 250 255

Val Leu Lys Gly His Asp Asp His Val Ile Thr Cys Leu Gln Phe Cys

260 265 270

Gly Asn Arg Ile Val Ser Gly Ser Asp Asp Asn Thr Leu Lys Val Trp

275 280 285

Ser Ala Val Thr Gly Lys Cys Leu Arg Thr Leu Val Gly His Thr Gly

290 295 300

Gly Val Trp Ser Ser Gln Met Arg Asp Asn Ile Ile Ile Ser Gly Ser

305 310 315 320

Thr Asp Arg Thr Leu Lys Val Trp Asn Ala Glu Thr Gly Glu Cys Ile

325 330 335

His Thr Leu Tyr Gly His Thr Ser Thr Val Arg Cys Met His Leu His

340 345 350

Glu Lys Arg Val Val Ser Gly Ser Arg Asp Ala Thr Leu Arg Val Trp

355 360 365

Asp Ile Glu Thr Gly Gln Cys Leu His Val Leu Met Gly His Val Ala

370 375 380

Ala Val Arg Cys Val Gln Tyr Asp Gly Arg Arg Val Val Ser Gly Ala

385 390 395 400

Tyr Asp Phe Met Val Lys Val Trp Asp Pro Glu Thr Glu Thr Cys Leu

405 410 415

His Thr Leu Gln Gly His Thr Asn Arg Val Tyr Ser Leu Gln Phe Asp

420 425 430

Gly Ile His Val Val Ser Gly Ser Leu Asp Thr Ser Ile Arg Val Trp

435 440 445

Asp Val Glu Thr Gly Asn Cys Ile His Thr Leu Thr Gly His Gln Ser

450 455 460

Leu Thr Ser Gly Met Glu Leu Lys Asp Asn Ile Leu Val Ser Gly Asn

465 470 475 480

Ala Asp Ser Thr Val Lys Ile Trp Asp Ile Lys Thr Gly Gln Cys Leu
485 490 495

Gln Thr Leu Gln Gly Pro Asn Lys His Gln Ser Ala Val Thr Cys Leu
500 505 510

Gln Phe Asn Lys Asn Phe Val Ile Thr Ser Ser Asp Asp Gly Thr Val
515 520 525

Lys Leu Trp Asp Leu Lys Thr Gly Glu Phe Ile Arg Asn Leu Val Thr
530 535 540

Leu Glu Ser Gly Gly Ser Gly Gly Val Val Trp Arg Ile Arg Ala Ser
545 550 555 560

Asn Thr Lys Leu Val Cys Ala Val Gly Ser Arg Asn Gly Thr Glu Glu
565 570 575

Thr Lys Leu Leu Val Leu Asp Phe Asp Val Asp Met Lys
580 585

<210> 9

<211> 559

<212> PRT

<213> Homo sapiens

<400> 9

Met Lys Ile Phe Ser Ile Ser Ile Ile Ala Gln Gly Leu Pro Phe Cys
1 5 10 15

Arg Arg Arg Met Lys Arg Lys Leu Asp His Gly Ser Glu Val Arg Ser
20 25 30

Phe Ser Leu Gly Lys Lys Pro Cys Lys Val Ser Glu Tyr Thr Ser Thr

35 40 45

Thr Gly Leu Val Pro Cys Ser Ala Thr Pro Thr Thr Phe Gly Asp Leu

50 55 60

Arg Ala Ala Asn Gly Gln Gly Gln Gln Arg Arg Arg Ile Thr Ser Val

65 70 75 80

Gln Pro Pro Thr Gly Leu Gln Glu Trp Leu Lys Met Phe Gln Ser Trp

85 90 95

Ser Gly Pro Glu Lys Leu Leu Ala Leu Asp Glu Leu Ile Asp Ser Cys

100 105 110

Glu Pro Thr Gln Val Lys His Met Met Gln Val Ile Glu Pro Gln Phe

115 120 125

Gln Arg Asp Phe Ile Ser Leu Leu Pro Lys Glu Leu Ala Leu Tyr Val

130 135 140

Leu Ser Phe Leu Glu Pro Lys Asp Leu Leu Gln Ala Ala Gln Thr Cys

145 150 155 160

Arg Tyr Trp Arg Ile Leu Ala Glu Asp Asn Leu Leu Trp Arg Glu Lys

165 170 175

Cys Lys Glu Glu Gly Ile Asp Glu Pro Leu His Ile Lys Arg Arg Lys

180 185 190

Val Ile Lys Pro Gly Phe Ile His Ser Pro Trp Lys Ser Ala Tyr Ile

195 200 205

Arg Gln His Arg Ile Asp Thr Asn Trp Arg Arg Gly Glu Leu Lys Ser

210 215 220

Pro Lys Val Leu Lys Gly His Asp Asp His Val Ile Thr Cys Leu Gln
225 230 235 240

Phe Cys Gly Asn Arg Ile Val Ser Gly Ser Asp Asp Asn Thr Leu Lys
245 250 255

Val Trp Ser Ala Val Thr Gly Lys Cys Leu Arg Thr Leu Val Gly His
260 265 270

Thr Gly Gly Val Trp Ser Ser Gln Met Arg Asp Asn Ile Ile Ile Ser
275 280 285

Gly Ser Thr Asp Arg Thr Leu Lys Val Trp Asn Ala Glu Thr Gly Glu
290 295 300

Cys Ile His Thr Leu Tyr Gly His Thr Ser Thr Val Arg Cys Met His
305 310 315 320

Leu His Glu Lys Arg Val Val Ser Gly Ser Arg Asp Ala Thr Leu Arg
325 330 335

Val Trp Asp Ile Glu Thr Gly Gln Cys Leu His Val Leu Met Gly His
340 345 350

Val Ala Ala Val Arg Cys Val Gln Tyr Asp Gly Arg Arg Val Val Ser
355 360 365

Gly Ala Tyr Asp Phe Met Val Lys Val Trp Asp Pro Glu Thr Glu Thr
370 375 380

Cys Leu His Thr Leu Gln Gly His Thr Asn Arg Val Tyr Ser Leu Gln
385 390 395 400

Phe Asp Gly Ile His Val Val Ser Gly Ser Leu Asp Thr Ser Ile Arg

405 410 415

Val Trp Asp Val Glu Thr Gly Asn Cys Ile His Thr Leu Thr Gly His

420 425 430

Gln Ser Leu Thr Ser Gly Met Glu Leu Lys Asp Asn Ile Leu Val Ser

435 440 445

Gly Asn Ala Asp Ser Thr Val Lys Ile Trp Asp Ile Lys Thr Gly Gln

450 455 460

Cys Leu Gln Thr Leu Gln Gly Pro Asn Lys His Gln Ser Ala Val Thr

465 470 475 480

Cys Leu Gln Phe Asn Lys Asn Phe Val Ile Thr Ser Ser Asp Asp Gly

485 490 495

Thr Val Lys Leu Trp Asp Leu Lys Thr Gly Glu Phe Ile Arg Asn Leu

500 505 510

Val Thr Leu Glu Ser Gly Gly Ser Gly Gly Val Val Trp Arg Ile Arg

515 520 525

Ala Ser Asn Thr Lys Leu Val Cys Ala Val Gly Ser Arg Asn Gly Thr

530 535 540

Glu Glu Thr Lys Leu Leu Val Leu Asp Phe Asp Val Asp Met Lys

545 550 555

<210> 10

<211> 540

<212> PRT

<213> Homo sapiens

<400> 10

Met Lys Arg Lys Leu Asp His Gly Ser Glu Val Arg Ser Phe Ser Leu
1   5   10   15

Gly Lys Lys Pro Cys Lys Val Ser Glu Tyr Thr Ser Thr Thr Gly Leu
            20   25   30

Val Pro Cys Ser Ala Thr Pro Thr Thr Phe Gly Asp Leu Arg Ala Ala
        35   40   45

Asn Gly Gln Gly Gln Gln Arg Arg Arg Ile Thr Ser Val Gln Pro Pro
    50   55   60

Thr Gly Leu Gln Glu Trp Leu Lys Met Phe Gln Ser Trp Ser Gly Pro
65   70   75   80

Glu Lys Leu Leu Ala Leu Asp Glu Leu Ile Asp Ser Cys Glu Pro Thr
                85   90   95

Gln Val Lys His Met Met Gln Val Ile Glu Pro Gln Phe Gln Arg Asp
            100   105   110

Phe Ile Ser Leu Leu Pro Lys Glu Leu Ala Leu Tyr Val Leu Ser Phe
        115   120   125

Leu Glu Pro Lys Asp Leu Leu Gln Ala Ala Gln Thr Cys Arg Tyr Trp
    130   135   140

Arg Ile Leu Ala Glu Asp Asn Leu Leu Trp Arg Glu Lys Cys Lys Glu
145   150   155   160

Glu Gly Ile Asp Glu Pro Leu His Ile Lys Arg Arg Lys Val Ile Lys
                165   170   175

Pro Gly Phe Ile His Ser Pro Trp Lys Ser Ala Tyr Ile Arg Gln His

180 185 190

Arg Ile Asp Thr Asn Trp Arg Arg Gly Glu Leu Lys Ser Pro Lys Val

195 200 205

Leu Lys Gly His Asp Asp His Val Ile Thr Cys Leu Gln Phe Cys Gly

210 215 220

Asn Arg Ile Val Ser Gly Ser Asp Asp Asn Thr Leu Lys Val Trp Ser

225 230 235 240

Ala Val Thr Gly Lys Cys Leu Arg Thr Leu Val Gly His Thr Gly Gly

245 250 255

Val Trp Ser Ser Gln Met Arg Asp Asn Ile Ile Ile Ser Gly Ser Thr

260 265 270

Asp Arg Thr Leu Lys Val Trp Asn Ala Glu Thr Gly Glu Cys Ile His

275 280 285

Thr Leu Tyr Gly His Thr Ser Thr Val Arg Cys Met His Leu His Glu

290 295 300

Lys Arg Val Val Ser Gly Ser Arg Asp Ala Thr Leu Arg Val Trp Asp

305 310 315 320

Ile Glu Thr Gly Gln Cys Leu His Val Leu Met Gly His Val Ala Ala

325 330 335

Val Arg Cys Val Gln Tyr Asp Gly Arg Arg Val Val Ser Gly Ala Tyr

340 345 350

Asp Phe Met Val Lys Val Trp Asp Pro Glu Thr Glu Thr Cys Leu His

355 360 365

Thr Leu Gln Gly His Thr Asn Arg Val Tyr Ser Leu Gln Phe Asp Gly

370 375 380

Ile His Val Val Ser Gly Ser Leu Asp Thr Ser Ile Arg Val Trp Asp

385 390 395 400

Val Glu Thr Gly Asn Cys Ile His Thr Leu Thr Gly His Gln Ser Leu

405 410 415

Thr Ser Gly Met Glu Leu Lys Asp Asn Ile Leu Val Ser Gly Asn Ala

420 425 430

Asp Ser Thr Val Lys Ile Trp Asp Ile Lys Thr Gly Gln Cys Leu Gln

435 440 445

Thr Leu Gln Gly Pro Asn Lys His Gln Ser Ala Val Thr Cys Leu Gln

450 455 460

Phe Asn Lys Asn Phe Val Ile Thr Ser Ser Asp Asp Gly Thr Val Lys

465 470 475 480

Leu Trp Asp Leu Lys Thr Gly Glu Phe Ile Arg Asn Leu Val Thr Leu

485 490 495

Glu Ser Gly Gly Ser Gly Gly Val Val Trp Arg Ile Arg Ala Ser Asn

500 505 510

Thr Lys Leu Val Cys Ala Val Gly Ser Arg Asn Gly Thr Glu Glu Thr

515 520 525

Lys Leu Leu Val Leu Asp Phe Asp Val Asp Met Lys

530 535 540

<210> 11

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
Oligonucleotide primer

<400> 11

cgggatccac catggatgat ggatcgatga cacc 34

<210> 12

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
Oligonucleotide primer

<400> 12

ggaattcctt aagggtatac agcatcaaag tcg 33

<210> 13

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
Oligonucleotide primer

<400> 13
tcacttcatg tccacatcaa agtcc 25

<210> 14
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide primer

<400> 14
ggtaattaca agttcttgtt gaactg 26

<210> 15
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide primer

<400> 15
ccctgcaacg tgtgtagaca gg 22

<210> 16
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:

Oligonucleotide primer

<400> 16

ccagtctctg cattccacac tttg 24

<210> 17

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

Oligonucleotide primer

<400> 17

ctcagacagg tcaggacatt tgg 23

<210> 18

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

Oligonucleotide primer

<400> 18

ggaattccat gaaaagattg gaccatggtt ctg 33

<210> 19

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
Oligonucleotide primer

<400> 19

ggaattcctc acttcatgtc acatcaaagt ccag 34

<210> 20

<211> 1881

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: 6 myc tagged
homo sapiens

<400> 20

atggagcaaa agctcatttc tgaagaggac ttgaatgaaa tggagcaaaa gctcatttct 60
gaagaggact tgaatgaaat ggagcaaaag ctcatttctg aagaggactt gaatgaaatg 120
gagcaaaagc tcatttctga agaggacttg aatgaaatgg agcaaaagct catttctgaa 180
gaggacttga atgaaatgga gagcttgggc gacctcacca tggagcaaaa gctcatttct 240
gaagaggact tgaattccat gaaaagaaag ttggaccatg gttctgaggt ccgctctttt 300
tctttgggaa agaaaccatg caaagtctca gaatatacaa gtaccactgg gcttgtacca 360
tgttcagcaa caccaacaac ttttggggac ctcagagcag ccaatggcca agggcaacaa 420
cgacgccgaa ttacatctgt ccagccacct acaggcctcc aggaatggct aaaaatgttt 480
cagagctgga gtggaccaga gaaattgctt gctttagatg aactcattga tagttgtgaa 540
ccaacacaag taaaacatat gatgcaagtg atagaacccc agtttcaacg agacttcatt 600
tcattgctcc ctaaagagtt ggcactctat gtgctttcat tcctggaacc caaagacctg 660
ctacaagcag ctcagacatg tcgctactgg agaattttgg ctgaagacaa ccttctctgg 720
agagagaaat gcaaagaaga ggggattgat gaaccattgc acatcaagag aagaaaagta 780
ataaaaccag gtttcataca cagtccatgg aaaagtgcat acatcagaca gcacagaatt 840
gatactaact ggaggcgagg agaactcaaa tctcctaagg tgctgaaagg acatgatgat 900
catgtgatca catgcttaca gttttgtggt aaccgaatag ttagtggttc tgatgacaac 960
actttaaaag tttggtcagc agtcacaggc aaatgtctga gaacattagt gggacataca 1020
ggtggagtat ggtcatcaca aatgagggac aacatcatca ttagtggatc tacagatcgg 1080

acactcaaag tgtggaatgc agagactgga gaatgtatac acaccttata tgggcatact 1140
tccactgtgc gttgtatgca tcttcatgaa aaaagagttg ttagcggttc tcgagatgcc 1200
actcttaggg tttgggatat tgagacaggc cagtgtttac atgttttgat gggtcatgtt 1260
gcagcagtcc gctgtgttca atatgatggc aggagggttg ttagtggagc atatgatttt 1320
atggtaaagg tgtgggatcc agagactgaa acctgtctac acacgttgca ggggcatact 1380
aatagagtct attcattaca gtttgatggt atccatgtgg tgagtggatc tcttgataca 1440
tccatccgtg tttgggatgt ggagacaggg aattgcattc acacgttaac agggcaccag 1500
tcgttaacaa gtggaatgga actcaaagac aatattcttg tctctgggaa tgcagattct 1560
acagttaaaa tctgggatat caaaacagga cagtgtttac aaacattgca aggtcccaac 1620
aagcatcaga gtgctgtgac ctgtttacag ttcaacaaga actttgtaat taccagctca 1680
gatgatggaa ctgtaaaact atgggacttg aaaacgggtg aatttattcg aaacctagtc 1740
acattggaga gtgggggggag tgggggagtt gtgtggcgga tcagagcctc aaacacaaag 1800
ctggtgtgtg cagttgggag tcggaatggg actgaagaaa ccaagctgct ggtgctggac 1860
tttgatgtgg acatgaagtg a 1881

<210> 21

<211> 626

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: 6 myc tagged
homo sapien

<400> 21

Met Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Glu Met Glu Gln
1 5 10 15

Lys Leu Ile Ser Glu Glu Asp Leu Asn Glu Met Glu Gln Lys Leu Ile
20 25 30

Ser Glu Glu Asp Leu Asn Glu Met Glu Gln Lys Leu Ile Ser Glu Glu
35 40 45

Asp Leu Asn Glu Met Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn

50 55 60

Glu Met Glu Ser Leu Gly Asp Leu Thr Met Glu Gln Lys Leu Ile Ser

65 70 75 80

Glu Glu Asp Leu Asn Ser Met Lys Arg Lys Leu Asp His Gly Ser Glu

85 90 95

Val Arg Ser Phe Ser Leu Gly Lys Lys Pro Cys Lys Val Ser Glu Tyr

100 105 110

Thr Ser Thr Thr Gly Leu Val Pro Cys Ser Ala Thr Pro Thr Thr Phe

115 120 125

Gly Asp Leu Arg Ala Ala Asn Gly Gln Gly Gln Gln Arg Arg Arg Ile

130 135 140

Thr Ser Val Gln Pro Pro Thr Gly Leu Gln Glu Trp Leu Lys Met Phe

145 150 155 160

Gln Ser Trp Ser Gly Pro Glu Lys Leu Leu Ala Leu Asp Glu Leu Ile

165 170 175

Asp Ser Cys Glu Pro Thr Gln Val Lys His Met Met Gln Val Ile Glu

180 185 190

Pro Gln Phe Gln Arg Asp Phe Ile Ser Leu Leu Pro Lys Glu Leu Ala

195 200 205

Leu Tyr Val Leu Ser Phe Leu Glu Pro Lys Asp Leu Leu Gln Ala Ala

210 215 220

Gln Thr Cys Arg Tyr Trp Arg Ile Leu Ala Glu Asp Asn Leu Leu Trp

225 230 235 240

Arg Glu Lys Cys Lys Glu Glu Gly Ile Asp Glu Pro Leu His Ile Lys
245 250 255

Arg Arg Lys Val Ile Lys Pro Gly Phe Ile His Ser Pro Trp Lys Ser
260 265 270

Ala Tyr Ile Arg Gln His Arg Ile Asp Thr Asn Trp Arg Arg Gly Glu
275 280 285

Leu Lys Ser Pro Lys Val Leu Lys Gly His Asp Asp His Val Ile Thr
290 295 300

Cys Leu Gln Phe Cys Gly Asn Arg Ile Val Ser Gly Ser Asp Asp Asn
305 310 315 320

Thr Leu Lys Val Trp Ser Ala Val Thr Gly Lys Cys Leu Arg Thr Leu
325 330 335

Val Gly His Thr Gly Gly Val Trp Ser Ser Gln Met Arg Asp Asn Ile
340 345 350

Ile Ile Ser Gly Ser Thr Asp Arg Thr Leu Lys Val Trp Asn Ala Glu
355 360 365

Thr Gly Glu Cys Ile His Thr Leu Tyr Gly His Thr Ser Thr Val Arg
370 375 380

Cys Met His Leu His Glu Lys Arg Val Val Ser Gly Ser Arg Asp Ala
385 390 395 400

Thr Leu Arg Val Trp Asp Ile Glu Thr Gly Gln Cys Leu His Val Leu
405 410 415

Met Gly His Val Ala Ala Val Arg Cys Val Gln Tyr Asp Gly Arg Arg
420 425 430

Val Val Ser Gly Ala Tyr Asp Phe Met Val Lys Val Trp Asp Pro Glu
435 440 445

Thr Glu Thr Cys Leu His Thr Leu Gln Gly His Thr Asn Arg Val Tyr
450 455 460

Ser Leu Gln Phe Asp Gly Ile His Val Val Ser Gly Ser Leu Asp Thr
465 470 475 480

Ser Ile Arg Val Trp Asp Val Glu Thr Gly Asn Cys Ile His Thr Leu
485 490 495

Thr Gly His Gln Ser Leu Thr Ser Gly Met Glu Leu Lys Asp Asn Ile
500 505 510

Leu Val Ser Gly Asn Ala Asp Ser Thr Val Lys Ile Trp Asp Ile Lys
515 520 525

Thr Gly Gln Cys Leu Gln Thr Leu Gln Gly Pro Asn Lys His Gln Ser
530 535 540

Ala Val Thr Cys Leu Gln Phe Asn Lys Asn Phe Val Ile Thr Ser Ser
545 550 555 560

Asp Asp Gly Thr Val Lys Leu Trp Asp Leu Lys Thr Gly Glu Phe Ile
565 570 575

Arg Asn Leu Val Thr Leu Glu Ser Gly Gly Ser Gly Gly Val Val Trp
580 585 590

Arg Ile Arg Ala Ser Asn Thr Lys Leu Val Cys Ala Val Gly Ser Arg
595 600 605

Asn Gly Thr Glu Glu Thr Lys Leu Leu Val Leu Asp Phe Asp Val Asp

610 615 620

Met Lys

625

<210> 22

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

Oligonucleotide primer

<400> 22

gggtacccct cattattccc tcgagttctt c 31

<210> 23

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

Oligonucleotide primer

<400> 23

ggaattcctt catgtccaca tcaaagtcc 29

<210> 24

<211> 2010

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: V5HIS tagged homo sapien

<400> 24

atgtgtgtcc cgagaagcgg tttgatactg agctgcattt gcctttactg tggagttttg 60
ttgccggttc tgctccctaa tcttcctttt ctgacgtgcc tgagcatgtc cacattagaa 120
tctgtgacat acctacctga aaaaggttta tattgtcaga gactgccaag cagccggaca 180
cacgggggca cagaatcact gaaggggaaa aatacagaaa atatgggttt ctacggcaca 240
ttaaaaatga ttttttacaa aatgaaaaga aagttggacc atggttctga ggtccgctct 300
ttttctttgg gaaagaaacc atgcaaagtc tcagaatata caagtaccac tgggcttgta 360
ccatgttcag caacaccaac aacttttggg gacctcagag cagccaatgg ccaagggcaa 420
caacgacgcc gaattacatc tgtccagcca cctacaggcc tccaggaatg gctaaaaatg 480
tttcagagct ggagtggacc agagaaattg cttgctttag atgaactcat tgatagttgt 540
gaaccaacac aagtaaaaca tatgatgcaa gtgatagaac cccagtttca acgagacttc 600
atttcattgc tccctaaaga gttggcactc tatgtgcttt cattcctgga acccaaagac 660
ctgctacaag cagctcagac atgtcgctac tggagaattt tggctgaaga caaccttctc 720
tggagagaga aatgcaaaga agaggggatt gatgaaccat tgcacatcaa gagaagaaaa 780
gtaataaaac caggtttcat acacagtcca tggaaaagtg catacatcag acagcacaga 840
attgatacta actggaggcg aggagaactc aaatctccta aggtgctgaa aggacatgat 900
gatcatgtga tcacatgctt acagttttgt ggtaaccgaa tagttagtgg ttctgatgac 960
aacactttaa aagtttggtc agcagtcaca ggcaaatgtc tgagaacatt agtgggacat 1020
acaggtggag tatggtcatc acaaatgaga gacaacatca tcattagtgg atctacagat 1080
cggacactca aagtgtggaa tgcagagact ggagaatgta tacacacctt atatgggcat 1140
acttccactg tgcgttgtat gcatcttcat gaaaaaagag ttgttagcgg ttctcgagat 1200
gccactctta gggtttggga tattgagaca ggccagtgtt tacatgtttt gatgggtcat 1260
gttgcagcag tccgctgtgt tcaatatgat ggcaggaggg ttgttagtgg agcatatgat 1320
tttatggtaa aggtgtggga tccagagact gaaacctgtc tacacacgtt gcaggggcat 1380
actaatagag tctattcatt acagtttgat ggtatccatg tggtgagtgg atctcttgat 1440
acatcaatcc gtgtttggga tgtggagaca gggaattgca ttcacacgtt aacagggcac 1500
cagtcgttaa caagtggaat ggaactcaaa gacaatattc ttgtctctgg gaatgcagat 1560
tctacagtta aaatctggga tatcaaaaca ggacagtgtt tacaaacatt gcaaggtccc 1620

aacaagcatc agagtgctgt gacctgttta cagttcaaca agaactttgt aattaccagc 1680
tcagatgatg gaactgtaaa actatgggac ttgaaaacgg gtgaatttat tcgaaaccta 1740
gtcacattgg agagtggggg gagtggggga gttgtgtggc ggatcagagc ctcaaacaca 1800
aagctggtgt gtgcagttgg gagtcggaat gggactgaag aaaccaagct gctggtgctg 1860
gactttgatg tggacatgaa ggaattctgc agatatccag cacagtggcg gccgctcgag 1920
tctagagggc ccttcgaagg taagcctatc cctaaccctc tcctcggtct cgattctacg 1980
cgtaccggtc atcatcacca tcaccattga 2010

<210> 25

<211> 669

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: V5HIS tagged
homo sapien

<400> 25

Met Cys Val Pro Arg Ser Gly Leu Ile Leu Ser Cys Ile Cys Leu Tyr
1 5 10 15

Cys Gly Val Leu Leu Pro Val Leu Leu Pro Asn Leu Pro Phe Leu Thr
20 25 30

Cys Leu Ser Met Ser Thr Leu Glu Ser Val Thr Tyr Leu Pro Glu Lys
35 40 45

Gly Leu Tyr Cys Gln Arg Leu Pro Ser Ser Arg Thr His Gly Gly Thr
50 55 60

Glu Ser Leu Lys Gly Lys Asn Thr Glu Asn Met Gly Phe Tyr Gly Thr
65 70 75 80

Leu Lys Met Ile Phe Tyr Lys Met Lys Arg Lys Leu Asp His Gly Ser

85 90 95

Glu Val Arg Ser Phe Ser Leu Gly Lys Lys Pro Cys Lys Val Ser Glu

100 105 110

Tyr Thr Ser Thr Thr Gly Leu Val Pro Cys Ser Ala Thr Pro Thr Thr

115 120 125

Phe Gly Asp Leu Arg Ala Ala Asn Gly Gln Gly Gln Gln Arg Arg Arg

130 135 140

Ile Thr Ser Val Gln Pro Pro Thr Gly Leu Gln Glu Trp Leu Lys Met

145 150 155 160

Phe Gln Ser Trp Ser Gly Pro Glu Lys Leu Leu Ala Leu Asp Glu Leu

165 170 175

Ile Asp Ser Cys Glu Pro Thr Gln Val Lys His Met Met Gln Val Ile

180 185 190

Glu Pro Gln Phe Gln Arg Asp Phe Ile Ser Leu Leu Pro Lys Glu Leu

195 200 205

Ala Leu Tyr Val Leu Ser Phe Leu Glu Pro Lys Asp Leu Leu Gln Ala

210 215 220

Ala Gln Thr Cys Arg Tyr Trp Arg Ile Leu Ala Glu Asp Asn Leu Leu

225 230 235 240

Trp Arg Glu Lys Cys Lys Glu Glu Gly Ile Asp Glu Pro Leu His Ile

245 250 255

Lys Arg Arg Lys Val Ile Lys Pro Gly Phe Ile His Ser Pro Trp Lys

260 265 270

Ser Ala Tyr Ile Arg Gln His Arg Ile Asp Thr Asn Trp Arg Arg Gly
275 280 285

Glu Leu Lys Ser Pro Lys Val Leu Lys Gly His Asp Asp His Val Ile
290 295 300

Thr Cys Leu Gln Phe Cys Gly Asn Arg Ile Val Ser Gly Ser Asp Asp
305 310 315 320

Asn Thr Leu Lys Val Trp Ser Ala Val Thr Gly Lys Cys Leu Arg Thr
325 330 335

Leu Val Gly His Thr Gly Gly Val Trp Ser Ser Gln Met Arg Asp Asn
340 345 350

Ile Ile Ile Ser Gly Ser Thr Asp Arg Thr Leu Lys Val Trp Asn Ala
355 360 365

Glu Thr Gly Glu Cys Ile His Thr Leu Tyr Gly His Thr Ser Thr Val
370 375 380

Arg Cys Met His Leu His Glu Lys Arg Val Val Ser Gly Ser Arg Asp
385 390 395 400

Ala Thr Leu Arg Val Trp Asp Ile Glu Thr Gly Gln Cys Leu His Val
405 410 415

Leu Met Gly His Val Ala Ala Val Arg Cys Val Gln Tyr Asp Gly Arg
420 425 430

Arg Val Val Ser Gly Ala Tyr Asp Phe Met Val Lys Val Trp Asp Pro
435 440 445

Glu Thr Glu Thr Cys Leu His Thr Leu Gln Gly His Thr Asn Arg Val

450 455 460

Tyr Ser Leu Gln Phe Asp Gly Ile His Val Val Ser Gly Ser Leu Asp
465 470 475 480

Thr Ser Ile Arg Val Trp Asp Val Glu Thr Gly Asn Cys Ile His Thr
485 490 495

Leu Thr Gly His Gln Ser Leu Thr Ser Gly Met Glu Leu Lys Asp Asn
500 505 510

Ile Leu Val Ser Gly Asn Ala Asp Ser Thr Val Lys Ile Trp Asp Ile
515 520 525

Lys Thr Gly Gln Cys Leu Gln Thr Leu Gln Gly Pro Asn Lys His Gln
530 535 540

Ser Ala Val Thr Cys Leu Gln Phe Asn Lys Asn Phe Val Ile Thr Ser
545 550 555 560

Ser Asp Asp Gly Thr Val Lys Leu Trp Asp Leu Lys Thr Gly Glu Phe
565 570 575

Ile Arg Asn Leu Val Thr Leu Glu Ser Gly Gly Ser Gly Gly Val Val
580 585 590

Trp Arg Ile Arg Ala Ser Asn Thr Lys Leu Val Cys Ala Val Gly Ser
595 600 605

Arg Asn Gly Thr Glu Glu Thr Lys Leu Leu Val Leu Asp Phe Asp Val
610 615 620

Asp Met Lys Glu Phe Cys Arg Tyr Pro Ala Gln Trp Arg Pro Leu Glu
625 630 635 640

Ser Arg Gly Pro Phe Glu Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly

645 650 655

Leu Asp Ser Thr Arg Thr Gly His His His His His His

660 665

<210> 26

<211> 2001

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: MYCHIS tagged homo sapiens

<400> 26

atgtgtgtcc cgagaagcgg tttgatactg agctgcattt gcctttactg tggagttttg 60

ttgccggttc tgctccctaa tcttcctttt ctgacgtgcc tgagcatgtc cacattagaa 120

tctgtgacat acctacctga aaaaggttta tattgtcaga gactgccaag cagccggaca 180

cacgggggca cagaatcact gaaggggaaa aatacagaaa atatgggttt ctacggcaca 240

ttaaaaatga ttttttacaa aatgaaaaga aagttggacc atggttctga ggtccgctct 300

ttttctttgg gaaagaaacc atgcaaagtc tcagaatata caagtaccac tgggcttgta 360

ccatgttcag caacaccaac aacttttggg gacctcagag cagccaatgg ccaagggcaa 420

caacgacgcc gaattacatc tgtccagcca cctacaggcc tccaggaatg gctaaaaatg 480

tttcagagct ggagtggacc agagaaattg cttgctttag atgaactcat tgatagttgt 540

gaaccaacac aagtaaaaca tatgatgcaa gtgatagaac cccagtttca acgagacttc 600

atttcattgc tccctaaaga gttggcactc tatgtgcttt cattcctgga acccaaagac 660

ctgctacaag cagctcagac atgtcgctac tggagaattt tggctgaaga caaccttctc 720

tggagagaga aatgcaaaga agaggggatt gatgaaccat tgcacatcaa gagaagaaaa 780

gtaataaaac caggtttcat acacagtcca tggaaaagtg catacatcag acagcacaga 840

attgatacta actggaggcg aggagaactc aaatctccta aggtgctgaa aggacatgat 900

gatcatgtga tcacatgctt acagttttgt ggtaaccgaa tagttagtgg ttctgatgac 960

aacactttaa aagtttggtc agcagtcaca ggcaaatgtc tgagaacatt agtgggacat 1020

acaggtggag tatggtcatc acaaatgaga gacaacatca tcattagtgg atctacagat 1080
cggacactca aagtgtggaa tgcagagact ggagaatgta tacacacctt atatgggcat 1140
acttccactg tgcgttgtat gcatcttcat gaaaaaagag ttgttagcgg ttctcgagat 1200
gccactctta gggtttggga tattgagaca ggccagtgtt tacatgtttt gatgggtcat 1260
gttgcagcag tccgctgtgt tcaatatgat ggcaggaggg ttgttagtgg agcatatgat 1320
tttatggtaa aggtgtggga tccagagact gaaacctgtc tacacacgtt gcaggggcat 1380
actaatagag tctattcatt acagtttgat ggtatccatg tggtgagtgg atctcttgat 1440
acatcaatcc gtgtttggga tgtggagaca gggaattgca ttcacacgtt aacagggcac 1500
cagtcgttaa caagtggaat ggaactcaaa gacaatattc ttgtctctgg gaatgcagat 1560
tctacagtta aaatctggga tatcaaaaca ggacagtgtt tacaaacatt gcaaggtccc 1620
aacaagcatc agagtgctgt gacctgttta cagttcaaca agaactttgt aattaccagc 1680
tcagatgatg gaactgtaaa actatgggac ttgaaaacgg gtgaatttat tcgaaaccta 1740
gtcacattgg agagtggggg gagtggggga gttgtgtggc ggatcagagc ctcaaacaca 1800
aagctggtgt gtgcagttgg gagtcggaat gggactgaag aaaccaagct gctggtgctg 1860
gactttgatg tggacatgaa ggaattctgc agatatccag cacagtggcg gccgctcgag 1920
tctagagggc ccttcgaaca aaaactcatc tcagaagagg atctgaatat gcataccggt 1980
catcatcacc atcaccattg a 2001

<210> 27

<211> 666

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: MYCHIS tagged
homo sapiens

<400> 27

Met Cys Val Pro Arg Ser Gly Leu Ile Leu Ser Cys Ile Cys Leu Tyr
1 5 10 15

Cys Gly Val Leu Leu Pro Val Leu Leu Pro Asn Leu Pro Phe Leu Thr
20 25 30

Cys Leu Ser Met Ser Thr Leu Glu Ser Val Thr Tyr Leu Pro Glu Lys

35 40 45

Gly Leu Tyr Cys Gln Arg Leu Pro Ser Ser Arg Thr His Gly Gly Thr

50 55 60

Glu Ser Leu Lys Gly Lys Asn Thr Glu Asn Met Gly Phe Tyr Gly Thr

65 70 75 80

Leu Lys Met Ile Phe Tyr Lys Met Lys Arg Lys Leu Asp His Gly Ser

85 90 95

Glu Val Arg Ser Phe Ser Leu Gly Lys Lys Pro Cys Lys Val Ser Glu

100 105 110

Tyr Thr Ser Thr Thr Gly Leu Val Pro Cys Ser Ala Thr Pro Thr Thr

115 120 125

Phe Gly Asp Leu Arg Ala Ala Asn Gly Gln Gly Gln Gln Arg Arg Arg

130 135 140

Ile Thr Ser Val Gln Pro Pro Thr Gly Leu Gln Glu Trp Leu Lys Met

145 150 155 160

Phe Gln Ser Trp Ser Gly Pro Glu Lys Leu Leu Ala Leu Asp Glu Leu

165 170 175

Ile Asp Ser Cys Glu Pro Thr Gln Val Lys His Met Met Gln Val Ile

180 185 190

Glu Pro Gln Phe Gln Arg Asp Phe Ile Ser Leu Leu Pro Lys Glu Leu

195 200 205

Ala Leu Tyr Val Leu Ser Phe Leu Glu Pro Lys Asp Leu Leu Gln Ala

210 215 220

Ala Gln Thr Cys Arg Tyr Trp Arg Ile Leu Ala Glu Asp Asn Leu Leu

225 230 235 240

Trp Arg Glu Lys Cys Lys Glu Glu Gly Ile Asp Glu Pro Leu His Ile

245 250 255

Lys Arg Arg Lys Val Ile Lys Pro Gly Phe Ile His Ser Pro Trp Lys

260 265 270

Ser Ala Tyr Ile Arg Gln His Arg Ile Asp Thr Asn Trp Arg Arg Gly

275 280 285

Glu Leu Lys Ser Pro Lys Val Leu Lys Gly His Asp Asp His Val Ile

290 295 300

Thr Cys Leu Gln Phe Cys Gly Asn Arg Ile Val Ser Gly Ser Asp Asp

305 310 315 320

Asn Thr Leu Lys Val Trp Ser Ala Val Thr Gly Lys Cys Leu Arg Thr

325 330 335

Leu Val Gly His Thr Gly Gly Val Trp Ser Ser Gln Met Arg Asp Asn

340 345 350

Ile Ile Ile Ser Gly Ser Thr Asp Arg Thr Leu Lys Val Trp Asn Ala

355 360 365

Glu Thr Gly Glu Cys Ile His Thr Leu Tyr Gly His Thr Ser Thr Val

370 375 380

Arg Cys Met His Leu His Glu Lys Arg Val Val Ser Gly Ser Arg Asp

385 390 395 400

Ala Thr Leu Arg Val Trp Asp Ile Glu Thr Gly Gln Cys Leu His Val

405 410 415

Leu Met Gly His Val Ala Ala Val Arg Cys Val Gln Tyr Asp Gly Arg

420 425 430

Arg Val Val Ser Gly Ala Tyr Asp Phe Met Val Lys Val Trp Asp Pro

435 440 445

Glu Thr Glu Thr Cys Leu His Thr Leu Gln Gly His Thr Asn Arg Val

450 455 460

Tyr Ser Leu Gln Phe Asp Gly Ile His Val Val Ser Gly Ser Leu Asp

465 470 475 480

Thr Ser Ile Arg Val Trp Asp Val Glu Thr Gly Asn Cys Ile His Thr

485 490 495

Leu Thr Gly His Gln Ser Leu Thr Ser Gly Met Glu Leu Lys Asp Asn

500 505 510

Ile Leu Val Ser Gly Asn Ala Asp Ser Thr Val Lys Ile Trp Asp Ile

515 520 525

Lys Thr Gly Gln Cys Leu Gln Thr Leu Gln Gly Pro Asn Lys His Gln

530 535 540

Ser Ala Val Thr Cys Leu Gln Phe Asn Lys Asn Phe Val Ile Thr Ser

545 550 555 560

Ser Asp Asp Gly Thr Val Lys Leu Trp Asp Leu Lys Thr Gly Glu Phe

565 570 575

Ile Arg Asn Leu Val Thr Leu Glu Ser Gly Gly Ser Gly Gly Val Val

580 585 590

Trp Arg Ile Arg Ala Ser Asn Thr Lys Leu Val Cys Ala Val Gly Ser

        595   600   605

Arg Asn Gly Thr Glu Glu Thr Lys Leu Leu Val Leu Asp Phe Asp Val

    610   615   620

Asp Met Lys Glu Phe Cys Arg Tyr Pro Ala Gln Trp Arg Pro Leu Glu

625   630   635   640

Ser Arg Gly Pro Phe Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn

                645   650   655

Met His Thr Gly His His His His His His

            660   665

1